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4387

The Oxidation of Glutathione in Human Erythrocytes— A Reversible Reaction.

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The observations here reported have been made while working on an adaptation for human blood of Tunnicliffe's[1] method for the determination of a reduced glutathione. A complete report of these studies will appear later.

The general plan of the experiments was as follows: a given specimen of human blood was saturated with CO_2, a sample or samples removed for estimation of reduced glutathione; the remaining blood was saturated with air, again sampled and the remainder saturated a second time with CO_2 and a portion taken for determination of reduced glutathione. Saturation with CO_2 or with air was accomplished by shaking the blood gently in a 250 cc. Erlenmeyer flask in a specially designed motor-driven apparatus as a stream of moist CO_2 or air was passed through the flask. Samples were removed with a 10 cc. Oswald-Folin pipette and allowed to run into 10% trichloracetic acid. The samples which were saturated with CO_2 were run into the acid contained in flasks in which the air had been replaced by CO_2. The trichloracetic acid filtrates were titrated with 0.001 N iodine solution to the starch end point and with 0.001 N thiosulphate to the disappearance of color, as suggested by Perlzweig and Delrue.[2] The excess of potassium iodide they suggest was

[1] Tunnicliffe, H. E., *Biochem. J.*, 1925, ix, 777.

[2] Perlzweig, W. A., and Delrue, G., *Biochem. J.*, 1927, xxi, 1416.

omitted. The nitroprusside reaction as an outside indicator has been used as a check on the starch indicator in a large number of determinations on human blood. When the nitroprusside end point is used the figures obtained are from 85% to 95% of those obtained with filtrates containing a higher concentration of reduced glutathione and poorer agreement when the concentration is less. It appears that the same substance is being measured by either of the indicators and starch is much more accurate in my hands.

Calculations were based on 100 cc. packed erythrocytes. The volume of packed cells dealt with in a given sample was estimated from accurate hematocrit readings. The glutathione content of human serum and of a few white cells present in the bloods studied (less than 2,000 per cm.) has been shown to be so low that it may be disregarded in these experiments.

TABLE I.

Experiment No.	First CO_2	Air	Second CO_2
1	104	75	102
	100	76	101
2	114	76	116
	119	76	114
3	75	49	77
	76	49	76
4	99	62	94
5	107	78	112

The variation in reduced glutathione content of human blood, in mg. per 100 cc. packed erythrocytes, when saturated with CO_2 or with air.

In Table I the content of reduced glutathione is shown in milligrams per 100 cc. packed erythrocytes. The content of reduced glutathione in the blood when saturated with CO_2 is seen to be from 40 to 55% greater than when saturated with air. The alternate oxidation and reduction of this part of the glutathione present may be repeated a number of times without any permanent change.

It is obvious that any method for the quantitative estimation of reduced glutathione in red blood cells which does not take into account the ease with which oxidation or reduction takes place may involve an error of as much as 50%.

The conversion by CO_2 of oxyhemoglobin of the erythrocytes is apparently accompanied by a conversion of a considerable part of the oxidized glutathione into reduced glutathione. This suggests that there might be an intimate interrelationship between these 2 substances which would be of broad physiological significance.

4388

A New Vibriothrix.

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From the Department of Tropical Medicine, Tulane University.

An organism has been recently isolated from human stool by me, the characters of which I will briefly describe.

Morphology.—In preparations made from the water condensation of recent glucose agar cultures the most diverse forms are seen: bacillus-like forms, curved vibrio-like forms, and very long spiral forms. In addition peculiar globular bodies of very different size, some very minute, others very large, being 3 or 4 microns in diameter, are present.

Motility.—The organism is motile.

Staining Reactions.—It is gram-negative, not acid-fast.

Cultural Characters and Biochemical Reactions.—Grows well on all ordinary laboratory media. On glucose agar and several other media the color of the growth is orange yellow. Serum is not liquefied. Gelatin seems to be very slowly liquefied. The organism does not produce gas in any sugar. In certain sugars it increases the alkalinity of the medium.

Pathogenicity.—The organism in all probability is not pathogenic. The subcutaneous inoculation into guinea pigs produces no symptoms.

Classification.—I believe this organism may be correctly placed in the genus *Vibriothrix,* a genus which I created in 1917 for an organism which I found in 1910 and which I described with various generic names, *Spirillum zeylanicum* 1910, *Vibrio zeylanicus* 1913,

VIBRIOTHRIX AURIANTICA.

Incubation period	Motility	Gram	Litmus Milk	Glucose	Levulose	Maltose	Galactose	Saccharose	Lactose	Mannitol	Glycerine	Inuline	Dulcitol	Rhamnose	Inositol	Adonitol	Arabinose	Amygdalin	Salicin	Sorbitol	Raffinose	Dextrin	Erythrite	Xylose	Indol	Gelatine	Serum
7	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+ Liq.	0
14		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0

0 = negative reaction, *viz.*, no liquefaction, no production of acidity.

Bacillus zeylanicus, Spirobacillus zeylanicus.[1] This genus, which might perhaps be placed in the family Nocardiaceae, is characterized by the following features: Mycelial articles thin, of very different shape, bacillary, vibrio-like, spirillum-like, at times club ended. Globular or pear-shaped bodies of very variable size may be present. Gram-negative, not acid-fast.

The new *Vibriothrix* I have described in this paper differs from *Vibriothrix zeylanica,* as the colonies of the latter are white. I propose for this new Vibriothrix the name *V. auriantica.*

4389

Mannitol Agar in the Differentiation of the Fungi of the Type Blastomyces.

ALDO CASTELLANI.

From the Tropical Department, Tulane University.

The mannitol medium used is ordinary agar to which 4% mannitol has been added. Large tubes are used, not the ordinary culture tubes employed for bacterial cultures. The tubes after inoculation are kept at 26° or in New Orleans at the temperature of the room for 3 weeks. Two to 3 weeks after inoculation certain blastomycoides fungi produce black pigmentation of the medium constantly, others occasionally, others never produce any pigmentation. To the first group belongs *Blastomycoides immitis,* to the second *Blastomycoides dermatitidis,* to the third *Blastomycoides tulanensis.*

4390

A Mannitol Fermenting Monilia.

ALDO CASTELLANI.

From the Department of Tropical Medicine, Tulane University.

A large number of monilias have been described in recent years, in fact more than 30 species of the genus Monilia are known. None ferments mannitol. A few weeks ago from a specimen of sputum

[1] Castellani and Chalmers, "Manual of Tropical Medicine," 3rd edition, Wood & Co., New York, p. 1068 (*Vibriothrix zeylanica* Castellani).

obtained from a case of chronic bronchitis I isolated a monilia which ferments mannitol.

Brief description of the monilia.—The fungus has the botanical characters of a monilia. It is gram-positive and not acid-fast. It does not liquefy gelatin or serum. The microscopical examination of glucose agar cultures reveals the presence of a large number of free yeastlike bodies and also of a certain amount of mycelium.

The most interesting feature of the fungus is that it ferments, with production of gas, mannitol. In addition it ferments, with production of gas, glucose, galactose, maltose, levulose and dextrin. It produces acidity in arabinose and xylose. For this new Monilia I suggest the name *Monilia mannitofermentans.*

4391

A New Strain of the Metadysentery Bacilli.

ALDO CASTELLANI.

From the Department of Tropical Medicine, Tulane University.

Some years ago I introduced the following classification of the dysentery bacilli: 1. Lactose not fermented, mannitol not fermented, *Bacillus dysenteriae* Shiga-Kruse. 2. Lactose not fermented, mannitol fermented (no gas), *Bacillus paradysenteriae* Collins, with its principal varieties: variety *Flexneri,* variety *Hissi-Russelli.* 3. Lactose fermented (no gas), mannitol fermented (no gas) or not fermented, Metadysentery group of bacilli. The Metadysentery group may be subdivided as I have shown in a previous publication[1] into 2 subgroups: *Bacillus ceylonensis* A subgroup; *Bacillus ceylonensis* B subgroup.

The Metadysentery bacilli of the *B. ceylonensis* A type produce acid in lactose very slowly, indol is not produced or only in very small amount, milk is clotted very slowly. The metadysentery organisms of the *Bacillus ceylonensis* B type produce acid in lactose rapidly and clot milk fairly quickly; indol is produced.

Recently from a case of acute dysenteric colitis, I have isolated a strain of metadysentery bacillus which is culturally and bio-chemically completely identical with *Bacillus ceylonensis* A, but it differs from organism was agglutinated by the patient's blood, but was not

[1] Castellani and Chalmers, *Annales de l'Institut Pasteur,* 1920; Castellani, *Am. J. Trop. Med.,* 1927, vii.

agglutinated by any dysentery, paradysentery, or metadysentery serum except in a dilution of 1 to 20: it does not absorb the agglutinins of any of the dysentery, paradysentery and metadysentery sera tried. For purposes of reference I propose calling it *B. ceylonensis* A, strain S, the name of the patient from whom it was recovered beginning with S.

4392

Correlation of Urinary Findings and Renal Pathology in Experimental Streptococcal Nephritis.

CHARLES W. DUVAL.

From the Department of Pathology, Tulane University, School of Medicine.

The relation of the urinary to the kidney changes in experimental nephritis is of particular interest since in man, with few exceptions, we have been unable to do more than conjecture the cause of the different stages of nephritis, and to assume that in certain infections the concomitant acute nephritis lays the foundation for chronic diffuse renal disease months and years afterwards. Likewise, in human nephritis it is very difficult to compare any particular derangement of kidney function with the structural renal changes incident thereto.

This paper reports upon the sequence of the structural renal changes in correlation with the abnormal urinary findings in dogs in whom nephritis has been induced with the toxic product of *Streptococcus scarlatinae.* The results are especially noteworthy inasmuch as the kidney changes were produced with a nephritic agent that commonly causes nephritis in man, and a closer analogy could be drawn with the human disease than is possible where improbable excitants of human nephritis, like uranium nitrate, have been employed. The experiment has also afforded the opportunity to study the relation and sequence of the renal changes, and to trace the progress of their anatomical development.

Since nephritis frequently occurs spontaneously in the dog, we have used only young animals whose urine over a period of 10 days to 2 weeks was free from albumen, casts and other abnormalities; and whose kidney function, as determined by the phenolsulphenolphthalein test, was within the normal limits. During the period of observation the animals were kept in metabolism cages to facilitate

TABLE I.

Dog 14. Fatal *Single* Intravenous injection. Urinary findings in experimental nephritis induced with Streptococcal "Lysate".

Date	Volume of Urine Excreted in 24 hours	Intravenous Injection Streptococcus "Lysate"	Albumen	Blood	Bile	Casts			Phenolsulphonphthalein %Excreted in 2 Hours	Remarks
						Hyalin	Fine Granular	Coarse Granular		
Apr. 6	500	12 cc.	0	0	0	0	0	0	84	1st injection.
7	400		++	+	+	++	++	++		Marked evidence of nephritis
8	340		++	+	+	+	+	+		Animal very sick.
9	500		+	0	+	+	+	0		Animal improved.
14	200		+	0	++	+	0	+		
22	210		+	+	++	+	+	+		Animal not eating.
24	300		++	++	+	++	++	+		Severe diarrhea.
25	200		++	+	++	++	++	++	62	Very weak and sick.
27										Animal found dead in cage.

Autopsy: Acute hemorrhagic glomerulo-nephritis.

TABLE II.

Dog 6. *Single* non-fatal intraperitoneal injection. Urinary findings in experimental nephritis induced with Streptococcal "Lysate".

Date	Volume of Urine Excreted in 24 hours	Intraperitoneal Injection Streptococcus "Lysate"	Albumen	Blood	Bile	Casts			Phenolsulphonphthalein %Excreted in 2 Hours	Remarks
						Hyalin	Fine Granular	Coarse Granular		
Feb. 22	600	10 cc.	0	0	0	0	0	0	76	1st injection; vomited immediately.
23	500		0	0	0	0	0	0		
24	200		+++	+	+	+	+	+		Animal very sick.
25	230		+++	0	+	+	+	+		
26	530		++	0	+	+	+	+		Animal eating again.
Mar. 3	375		+	0	+	+	+	+		
4	300		+	+	+	+	0	+		
9	170		+	+	+	0	0	0		
16	210		+	0	+	+	+	+	64.5	
26	210		0	0	0	0	0	+		
28	90		+	0	+	0	0	0		
April 8	200		+	0	+	+	+	0		
15	185		+	0	+	+	+	+		
22	300		+	0	+	+	+	+	61.7	P S P Test shows reduced function.
28	280		+	0	+	+	+	+		
May 10	300		+	0	+	+	+	+		
20	330		++	0	+	++	++	++		
22	290		++	0	+	++	++	+		
28	180		+	0	+	++	++	++		
June 2	380		++	0	0	++	++	++		Evidence of Chr. Nephritis.

Animal killed November 20, six months after inoculation, at the time apparently in good health.
Autopsy: Chronic interstitial nephritis.

the collection of urine. Catheterized specimens were not employed, so the figures for urine volumes are averages only, obtained by dividing the total urine volume of 7 days by the number of days in the period. Daily examinations of urine were made for abnormalities over a period of from one to 3 weeks after the administration of the nephrotoxic substance (killed culture and "Lysate" which had been prepared *in vivo* from the *Streptococcus scarlatinae* by the method of Duval and Hibbard[1]). Of dogs who survived several months, urine examinations were made at weekly intervals after the first 3 weeks. The P.S.P. test for kidney function was repeatedly carried out on all dogs at approximately weekly intervals.

Fourteen young healthy dogs were employed. Two methods of inducting the nephritis were resorted to, namely: intraperitoneal and intravenous injections of "killed" culture and "Lysate" of *Streptococcus scarlatinae* respectively. The dosage of "killed" culture was the entire 48 hour surface growth of 3 blood-agar slants suspended in 15 cc. of normal sterile saline; while the "Lysate" dose was 10 cc. to 15 cc. of sterile Berkefeld "N" filtered peritoneal fluid from the immune rabbit that had previously received intraperitoneally a 48 hour streptococcal surface growth of 12 blood-agar slants.

It is noteworthy that the nephritic histopathology in all 14 animals corresponds to that of the various kidney lesions in scarlatina. The "killed" culture acted in the same manner as the "Lysate" in the production of nephritis; and regardless of whether the nephrotoxic substance was introduced intraperitoneally or intravenously.

In regard to nephritis all dogs employed in the experiment reacted to the streptococcal product in a characteristic manner. The nephritic effect was primarily upon the glomeruli, which frequently was hemorrhagic in character, as shown by the urine findings and histopathology. Four animals died in 3 to 4 days after the first injection of the "Lysate", and apparently the result of an acute hemorrhagic glomerulo-nephritis. The remaining 12 dogs of the series lived through the entire period of observation (1 to 6 months); however, they were all quite sick from 1 to 3 days following each injection, as was manifested by vomiting, anorexia, fever and the abnormal urine findings.

The urine findings from day to day were a fairly reliable index to the anatomical changes occurring in the kidneys as revealed by the histopathological study. Blood in the urine invariably meant glomerular hemorrhage, while fine granular casts foretold serious retrograde changes in the epithelium of the convoluted tubules which

[1] Duval, C. W., and Hibbard, R. J., *J. Exp. Med.*, 1926, xliv, 567.

seemed to be dependent upon, and always secondary to, lesions of an obstructive character in the capillary tufts of the glomeruli. The primary glomerular followed by secondary tubular lesions is the reverse picture of the parenchymatous nephritis in dogs induced with uranium nitrate which, according to Mac Nider,[2] produces a primary lesion of the epithelial cells of the proximal convoluted tubules.

The appearance of albumen and casts in the urine was a constant happening in all the injected dogs. In most cases albumen and casts occurred in 24 hours after the injection of the nephrotoxic agent, while in others, these abnormalities were found only after 2 to 3 days. In addition to albumen and casts the majority of dogs showed quantities of blood and bile in the urine, though usually these abnormal substances did not appear before the third day following the injection.

Four of the acute nephritic dogs were allowed to live for 6 months or longer. These animals in 2 or 3 months after the last injection of "Lysate" apparently returned to a normal renal function as indicated by the total absence of abnormal constituents in the urine. However, at autopsy there was very definite gross evidence of chronic renal changes which was confirmed by the microscopic study.

The results of the experiment as a whole show that the toxic product of *Streptococcus scarlatinae* whether introduced intraperitoneally or intravenously has a selective affinity for the glomeruli of the kidney, affecting primarily the capillary tufts (hemorrhagic glomerulonephritis). Secondary changes of a retrograde character occur for the tubular epithelium, particularly the epithelium of the convoluted tubules. It appears that regeneration of the tubular epithelium does not occur where the corresponding glomerular tuft is destroyed.

4393

The Heart of the Thoroughbred Race Horse. Studies in Hypertrophy.

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The interesting note in Sir William Osler's "Textbook of Medicine" on the heart of Eclipse, the famous race horse, and Master

[2] Mac Nider, Wm. de B., *J. Exp. Med.*, 1929, xlix, 387.

McGrath, the famous greyhound, has prompted investigation of the heart-weight body-weight ratios in these animals. Studies on the heart of the racing greyhound were reported in the PROCEEDINGS of 3 years ago.[1] The questions of true physiologic as well as the mechanism of pathologic work hypertrophy of the heart are still open.

We, unfortunately, do not have records of the heart-weight body-weight ratios of the great sires of thoroughbred racing stock or of the great mares that have produced long lines of winners. Accurate anatomical data on the English sires, or the lines descending from Godolphin Bart, 1689, Byerly Turk, 1706, and Darley Arabin, 1724, or of the American progenitor, Longfellow I, 1850, or the more recently famous studs, as Fair Play, Broomstick, and Man-of-War, would be most interesting and valuable.

In a group of 16 race horses of various strains, ages, and racing experiences, I have been able to obtain the heart-weight body-weight ratios and the left ventricle to right ventricle ratios. The greatest H.W./B.W. ratio, that of 110 gm. of heart per kilo of body weight, was gotten from Bronston, a horse that had been frequently "hopped" and had dropped dead during a morning workout. This horse may well have had some abnormality in his cardiovascular system.

The next highest heart weight ratio, that of 107, was found in Clorinda F, a 3-year-old, whose heart-weight body-weight ratio was actually greater than those of 3 and 4 year old horses. A note from the veterinarian stated that Clorinda F was of unusually good lineage.

Other than this the horses ranked in heart-weight body-weight ratios somewhat according to their age, the 4-year-olds having a ratio of 101 and 104, the 3-year-olds 82 to 100, and the 2-year-olds 81 to 88. The yearlings that had not been entered in competitive races averaged 71 to 84. This latter figure is considerably above that of 67.7 which is generally given for the heart-weight body-weight in horses. Thus even the thoroughbred colts apparently have comparatively larger hearts than the regular run of horses. The average of the whole series of race horses is .00925. The L/R ratios indicate that the hypertrophy is general, affecting the right ventricle as much or perhaps slightly more than it does the left heart.

These data suggest that the thoroughbred race horse has a larger heart to begin with and one that has a greater capacity for hypertrophy. The heart, furthermore, apparently responds to schooling

[1] Herrmann, George, PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 856.

and training by hypertrophying to an unusual degree. However, the race horse averages are far below those that I found in the greyhound series, and as far as my studies are concerned the greyhound still tops the list of heart-weight body-weight ratios for mammals.

The interesting question of attributing the findings to the inheritance of acquired characteristics or to the result of selection down through generations of racing stock is again raised.

New York Meeting.

New York Academy of Medicine, April 17, 1929.

4394

An Attempt to Secure "Refection" in Rats.*

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(With the Collaboration of Helen C. Cannon.)

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Fridericia[1] reported to the XII International Physiological Congress at Stockholm a phenomenon of apparent acquired immunity to deficiency of vitamin B in rats receiving a diet devoid of this vitamin group, but presumed to be adequate in all other respects. He stated that "In spite of the deprivation of B-vitamin a few of the rats resumed their normal rate of growth, and at the same time a change occurred in their intestinal tract, manifesting itself by their feces turning white and bulky. Eating of feces was prevented (wiregauze bottom in cages). Control rats, also fed on a diet devoid of B-vitamin, behaved in the usual way (growth stopping after 1 to 3 weeks, death after 4 to 5 weeks)."

To the restoration phenomenon Fridericia gave the name "refection". Subsequently he[2] described its manifestations in detail including the characteristic high content of undigested uncooked rice starch in the "white" feces. The hypothesis was offered that multiplication of micro-organisms supplying vitamin B occurs in "refected" rats; hence the unexpected resumption of growth. By feeding the feces of "refected" individuals to other rats that were showing failure of growth on a B-free diet, "refection" could often

* The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[1] Fridericia, L. S., XII Internat. Physiol. Congress at Stockholm, Aug. 3-6, 1926; *Skandin. Arch.*, 1926, xlix.

[2] Fridericia, L. S., Freudenthal, P., Gudjonnsson, S., Johansen, G., and Schoubye, N., *J. Hyg.*, 1927, xxvii, 70.

be induced and growth restored. The feces then always turned "white". Many additional surprising details have been recorded.

The "spontaneous cures" in rats reared on diets devoid of the vitamin B components have also been recorded by Roscoe[3] from the Lister Institute in London. However, "refection" did not occur there when the diet was cooked or the starch was replaced by a soluble carbohydrate. Workers[4] in the Cambridge Biochemical Laboratory likewise have reported that from time to time rats on a vitamin B-free diet would decline for a short time only, and then resume growth, and continue to grow at a nearly or quite normal rate. The customary diet there used contained rice starch and cane sugar as the carbohydrate components. In each case where the rats failed to show signs of vitamin B deficiency, the rice starch had been replaced by potato starch. Older animals were not "refected" as readily as young rats weighing 40-60 gm. Kon and Watchorn remark that apparently what is of occasional occurrence when the vitamin B-free diet contains rice starch, becomes almost the rule when potato starch is used. Raw arrowroot starch gave similar though less striking "refection" results. The protective action, in the Cambridge experiments, was largely destroyed by gentle cooking of the starch, and less so by extraction with alcohol.

In hundreds of experiments with white albino rats in the laboratories of both the Connecticut Agricultural Experiment Station and Yale University over a period of many years we have failed to note any comparable phenomenon of "spontaneous growth" with production of bulky white feces when the animals were living on diets rich in raw starch and devoid of the plurality of food factors formerly described as vitamin B. The customary ration, which varied from time to time in the proportions of the ingredients according to the purpose of the tests, had a composition essentially as follows: protein 18 to 35%, Osborne and Mendel salt mixture IV[5] 4%, fat 24%, and the remainder corn starch (Duryea brand). Cod liver oil was fed apart from this mixture, or else butter fat was included therein to supply fat-soluble vitamin. Latterly we have added small doses of irradiated ergosterol.

Years ago we observed that rats, maintained a long time on mixtures of isolated foodstuffs, often ate their own feces. Feeding small quantities of rat feces to animals showing nutritive decline sometimes brought about a favorable response in restoration of

[3] Roscoe, Margaret Honora, *J. Hyg.*, 1927, xxvii, 103.

[4] Kon, S. K., and Watchorn, Elsie, *J. Hyg.*, 1928, xxvii, 321.

[5] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, xxxvii, 572.

weight.[6] After the development of the vitamin hypothesis in the study of nutrition the favorable effects of feces were attributed to the presence of residual vitamins therein, perhaps of bacterial origin. The possibility of such a source is now generally recognized[7] and has led to the use of special cage devices whereby access to the feces is prevented.

The significance of the "refection" phenomena reported abroad for all experiments involving biological examinations for vitamin B in foods or other chemical substances is obvious. We have considered it worth while to make observations on the possibility of "refection" in our laboratory by imitating somewhat closely the dietary conditions reported by Kon and Watchorn, in contrast to our own customary feeding procedures. Two food mixtures were prepared with the same ingredients except that in one (Ration C) the raw starch was a commercial corn starch (Duryea brand), whereas the other (Ration P) contained starch prepared in the laboratory from potatoes. These rations devoid of added sources of vitamin B (*i. e.*, the components in yeast) contained casein 18%, starch 54%, Osborne and Mendel salt mixture[5] 4%, butter fat 9%, lard 15%. Ten male rats weighing 50-60 gm. were placed in individual cages with a fresh supply of water daily. Six of the animals received Ration P. Of these, 4 promptly declined in weight and died within a short time in the characteristic way. Another lived somewhat longer without substantial gain in weight. The sixth rat, B6963, on the potato starch diet grew at a slow rate during 40 days, when a decline ensued. Subsequently the animal maintained its weight for more than 2 months. The feces were noticed after one week to be lighter in color than usual, but not "white". Four rats received Ration C. Of these all declined in weight and developed polyneuritic symptoms or died within the customary period. Two of them, when nearly moribund, were fed feces secured from Rat B6963. These animals survived for some time with slight if any gains in weight.

Dr. Gudjonnsson of Professor Fridericia's laboratory observed Rat B6963 and its records during a visit here and expressed the

[6] Detailed bacteriological experiments with some of our animals were reported by Rettger, L. F., and Horton, G. D., *Centralbl. f. Bakteriol., I. Abt., Originale*, 1914, lxxiii, 362.

[7] Steenbock, H., Sell, Mariana T., and Nelson, E. M., *J. Biol. Chem.*, 1923, lv, 399; Dutcher, R. A., and Francis, Emma, PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 189; Bechdel, S. I., Honeywell, Hannah E., Dutcher, R. A., and Knutsen, M. H., *J. Biol. Chem.*, 1928, lxxx, 231; Smith, A. H., Cowgill, G. R., and Croll, Hilda M., *J. Biol. Chem.*, 1925, lxvi, 15.

opinion that there was no evidence of "refection". It has been suggested that potato starch at times may carry traces of vitamin B which would explain the delayed symptoms in one of the animals.

These results are indicative of the failures to observe in our laboratories phenomena comparable to "refection".

4395

Effect of Thyroparathyroidectomy on the Action of Irradiated Ergosterol.

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In a recent publication[1] we suggested that irradiated ergosterol increased the calcium concentration of the blood by acting on the parathyroid glands. This view was based on the following observation: Latent tetany, manifested by calcium concentration of about 6 mg. per 100 cc. of serum, was first brought about in a monkey by means of a low calcium diet. It was then found possible to raïse the calcium level to about 11 mg. by means of large amounts of irradiated ergosterol given by mouth. However, after extirpation of the parathyroid glands, the calcium could not be elevated much above 7 mg. per 100 cc. of serum. Slight hypercalcemia was induced by means of injections of parathyroid extract (Collip).

Urechia and Popoviciu[2] have given irradiated ergosterol to dogs in which tetany had been induced by means of parathyroidectomy. They concluded that the tetany was not relieved by this means nor the calcium level raised. They do not state the exact composition of the dietary. Brougher[3] carried out similar experiments. He concluded that irradiated ergosterol "when given in conjunction with milk from the day of operation prevents the development of violent tetany."

During the past year we have carried out numerous extirpation experiments on monkeys and on dogs with the same object in view. In many instances the technic has been modified by giving irradiated ergosterol at the outset in order to establish the fact that hypercalcemia could be induced; levels of 13-16 mg. per 100 cc. of serum

[1] Hess, A. F., Lewis, J. M., and Rivkin, H., *J. Am. Med. Assn.*, 1928, xci, 783.

[2] Urechia, C. I., and Popoviciu, G., *Compt. rendu. de la Soc. Biol.*, 1928, xcviii, 405.

[3] Brougher, J. C., *Am. J. Physiol.*, 1928, lxxxvi, 538.

were brought about in this way. Following operation, however, we were not able to raise the calcium above the normal level. It should be added that after large doses of irradiated ergosterol has been given for a period of some weeks, removal of the parathyroid glands does not result in the precipitous fall in the calcium which we are accustomed to observe following extirpation. This distinction is due probably to a storing of the calcium in the tissues. The results of this series of experiments may be summarized by the statement that our preliminary observation has been substantiated, in that hypercalcemia could not be induced or the calcium level elevated markedly, after the parathyroids had been removed.

Irradiated ergosterol was found to increase not only the calcium concentration but that of the inorganic phosphorus in the serum. The phosphorus rose frequently to a level of 12 to 14 mg. per 100 cc. of serum several days after irradiated ergosterol had been given. In this respect the action of irradiated ergosterol differs from that of parathyroid extract, which does not bring about an increase of inorganic phosphorus until hypercalcemia is marked and the animal is in a highly weakened condition. This effect may not be associated with the activity of the parathyroid glands. It should be mentioned also that the hypercalcemia brought about by irradiated ergosterol is far more prolonged, both in man and in animals, than that which follows injections of parathyroid extract.

The mechanism of the action of irradiated ergosterol is not entirely clear, but these experiments seem to be of interest as demonstrating that a vitamin may function, at least to a certain extent, through the medium of one of the endocrine glands.

4396

Attempts to Cultivate Vaccine Virus in the Growing Chick Embryo.

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The propagation of vaccine virus in the skin and cornea of susceptible animals has been supplemented by evidence of mass growth in the testicle (Noguchi[1]) and perhaps the brain (Levaditi[2]). At-

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[1] Noguchi, *J. Exp. Med.*, 1915, xxi, 565; xxvii, 423.

[2] Levaditi, Harvier and Nicolau, *Compt. Rend. Soc. Biol.*, 1921, lxxxv, 345.

tempts to cultivate vaccine virus elsewhere than in the skin of living animals have as their objectives purification of the virus, the production of larger amounts at less cost as well as to throw light on the nature of the virus itself.

These objectives would all be reached and extended if it were possible to grow vaccine virus outside the animal body in ordinary or in tissue culture. It is the generally accepted opinion that viruses in general will increase only in the presence of living cells (Rivers, p. 13[3]) for which they have specific affinity.

The claims of Fornet[4] and of Volpino[5] of having grown vaccine virus in serum in the presence of sugar or glycerine have not been confirmed. The addition of animal tissue to such media with positive results in the hands of Pröscher[6] and of Plotz[7] has not only been unconfirmed but is denied by Gins.[8] The remarkable results recently claimed by Maitland and Maitland,[9] who apparently obtained marked increase of the virus in a series of cultures comprising minced hen kidney, hen serum and Tyrode's solution, and grown aerobically certainly inspire repetition.

Distinct evidence of the growth of vaccine virus in tissue cultures of adult corneal epithelium (Steinhardt, Israel and Lambert[10]; Gins[11]) and in testicular extract (Flores Cordova[12]) or testicular cells (Parker[13]; Parker and Nye[14]; Minervin and Schmerling[15]; Haagen[16]) has been generally accepted. Even more interesting positive results in tissue culture with embryonic rabbit and guinea pig cornea were obtained by Cracium and Oppenheimer.[17] Carrel and Rivers[18] in tissue cultures from the skin, cornea and brain of em-

[3] Rivers, "Filterable Viruses," 1928, Williams & Wilkins.

[4] Fornet, *Berlin. Klin. Wochenschr.*, 1913, 1864. *Centralbl. Bakt.*, 1922, lxxxvii, 36.

[5] Volpino, *Pathologica*, 1921, xiii.

[6] Pröscher, *Centralbl. Bakt.*, 1906, xl, 337. *Berlin. Klin. Wochenschr.*, 1915, 886.

[7] Plotz, *Compt. Rend. hebdom. des seances de l'Acad. des Sciences*, 1922, clxxiv, 1265.

[8] Gins, *Berlin. Klin. Wochenschr.*, 1913, 2349; 1914, 391. *Z. f. Hyk.*, 1916 lxxxii, 98.

[9] Maitland and Maitland, *Lancet*, 1928, ii, 596.

[10] Steinhardt, Israel and Lambert, *J. Inf. Dis.*, 1913, xiii, 294.

[11] Gins, *Z. f. Hyg.*, 1916, lxxxii, 101.

[12] Flores Cordova, *Cronica Medica*, 1926, xliii, 377.

[13] Parker, *J. Med. Res.*, 1924, xliv, 645.

[14] Parker and Nye, *Am. J. Path.*, 1925, i, 325.

[15] Minervin and Schmerling, *Centralbl. Bakt.*, 1926, xcix, 558.

[16] Haagen, *Centralbl. Bakt.*, 1928, cix, 31.

[17] Cracium and Oppenheimer, *J. Exp. Med.*, 1926, xliii, 815.

[18] Carrel and Rivers, *Compt. Rend. Soc. Biol.*, 1927, xcvi, 848.

bryo chicks succeeded in increasing 25 vaccine units to 10,000 in 10 days. These last results have been confirmed by Levaditi[19] with his neuro-vaccine, and by Eagles and McLean.[20]

It occurred to us that since tissues of the embryonic chick in culture appear to furnish the most suitable medium for the growth of vaccine virus, a technically simpler and possibly better result might be obtained by direct inoculation of fertile and incubating hens' eggs. It was first ascertained that egg white is distinctly destructive for vaccine virus when incubated with proper control at 37° C. for 24 hours, whereas egg yolk is apparently harmless. The virus inoculated in the yolk of an infertile egg survives in the incubator for 4 but not for 8 days.

Fertile hens' eggs in a number of different lots were incubated at 40° C. for from 5 to 6 days and were then inoculated aseptically in the yolk with the Noguchi strain of rabbit testicle vaccine-virus. The incubation was continued for from 4 to 10 days, the eggs were opened after treating with alcohol and the free flame, or else the yolk withdrawn through a freshly sterilized area of the shell. The embryos examined at successive stages after introduction of the virus in the yolk showed first a marked congestion of the vessels followed by a shrinking of the embryo which was then found to be dead and fragile. In some instances, of course, bacterial contamination occurred. In nearly every sterile although usually dead embryo up to 10 days after inoculation (total incubation 15 to 16 days; length of embryo up to 30 mm.) living virus was found as tested intradermally in the rabbit skin. There was more virus present in the embryo than in yolk per unit of volume, and more virus than in fresh vaccine diluted with equal parts of ground embryo of the same age. In other words, there was distinct evidence of multiplication of the virus and of its concentration in the embryo from the yolk. A second series of incubated eggs was inoculated in the yolk from the first incubated yolk, and the virus was still present several days later. A third transfer was negative. In such series experiments, transfers from the embryonic tissue which shows greater amounts and increase of virus in the first series, although technically more difficult, might well have yielded more favorable results.

Direct inoculation of vaccine virus into the yolk of fertile, incubated hens' eggs gives evidence, then, of some increase in the virus but leads to the death of the embryo in a few days. It is still pos-

[19] Levaditi, *Compt. Rend. Soc. Biol.*, 1927, xcvi, 967.

[20] Eagles and McClean, *Brit. J. Exp. Path.*, 1929, xvi, 35.

sible that by modifying the conditions of these experiments a new method of cultivating vaccine virus may be obtained.

4397

Attempted Production of Vaccinal Encephalitis in Rabbits with a Testicular Virus.

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The production of vaccinal encephalitis in rabbits is of interest at this time chiefly in view of the prevalence of post vaccinal encephalitis in some European countries. The possible use of the rabbit's brain as a medium for mass production of sterile vaccine (advocated by Levaditi) also adds importance to the question.

A considerable number of European workers have succeeded in producing what they regard as a vaccinal encephalitis in rabbits. Levaditi[1] and his co-workers since 1921 have developed a strain of vaccine virus, known as neurovaccine, which produces regularly on intracerebral injection into rabbits a typical encephalitis with paralytic symptoms and death in 4-7 days. They regard it as a virus adapted to the central nervous system by passage and consider that it has acquired neurotropic properties. They first adapted it to the brain by alternate brain and testicular passage—later omitting the testicular passages. Blanc and Caminopetros[2] found that ordinary calf vaccine passed through the rabbit's cornea and then to the brain was sufficiently adapted to cause a fatal encephalitis. Herzberg[3] produced a vaccinal encephalitis by the intracerebral injection of a virus adapted to the rabbit's testicle.

The great majority of workers, however, have found no adaptation necessary and consider the encephalitogenic property as one inherent in ordinary vaccine virus. Marie,[4] Krumbach,[5] Condrea,[6]

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1 Levaditi, Harvier and Nicolau, *Compt. Rend. Soc. Biol.*, 1921, lxxxv, 345; Levaditi and Nicolau, *Compt. Rend. Soc. Biol.*, 1922, lxxxvi, 77; Levaditi, *J. State Med.*, 1924, xxxii, 151.

2 Blanc and Caminopetros, *Compt. Rend. Soc. Biol.*, 1923, lxxxviii, 1020.

3 Herzberg, *Cent. f. Bakt.*, 1925, xcv, 211.

4 Marie, *Compt. Rend. Soc. Biol.*, 1920, lxxxiii, 476.

5 Krumback, *Z. f. Immuntatsforsch.*, 1922, xxxviii, 1.

6 Condrea, *Compt. Rend. Soc. Biol.*, 1922, lxxxvi, 897.

Bachman and Bigliere,[7] Burnet and Conseil,[8] Winkler,[9] have all succeeded in producing an encephalitis by the intracerebral injection of ordinary calf vaccine lymph purified by various means. Krumbach found no difference between calf lymph and lapine (vaccinia adapted to rabbit's skin by passage). Condrea was unable to detect any difference between the action of cutaneous and testicular virus in the brain—both producing encephalitis. Bachman and Biglieri used 4 strains of vaccinia and one of variola and obtained identical symptoms with all (although the symptoms described by them differ markedly from the typical picture described by most other authors). They also found no difference between testicular and dermal virus. Burnet and Conseil found that chloral or opium injections increased the susceptibility of the brain. Ledingham[10] states that ordinary vaccine virus requires no special adaptation to the brain to kill by intracerebral injection.

The reports of failures to produce encephalitis by vaccine virus are comparatively rare. Calmette and Guerin[11] injected virus intracerebrally and recovered it from the brain the fourth but not the seventh day—but make no mention of any symptoms or fatal disease. Camus[12] was unable to produce encephalitis in rabbits by the intracerebral injection of pure vaccine and on the basis of this and the difference between the skin lesions of ordinary vaccine and Levaditi's neurovaccine is inclined to regard Levaditi's product as a mixture of vaccine virus with some other unknown virus. Walthard[13] could not produce encephalitis by the injection into one rabbit of virus deposited in the brain of another after corneal infection.

So far as is known there is no report in the American literature on the production of encephalitis by an ordinary strain of vaccine virus—either by direct injection or with any means of adaptation. (Although Levaditi's neurovaccine has been used by a number of workers.) In view of this it was considered worth while to endeavor to produce an encephalitis in rabbits by using a strain not known to be adapted to the brain. The virus used is the testicular strain adapted by Noguchi originally and supplied to us by Dr. T. M. Rivers. Injection into the 4th ventricle was used at first because of its simplicity and its very successful use with herpes encepha-

[7] Bachman and Biglieri, *Compt. Rend. Soc. Biol.*, 1923, lxxxviii, 351.

[8] Burnet and Conseil, *Compt. Rend. Soc. Biol.*, 1924, xc, 1408.

[9] Winkler, *Ergeb. der Hygiène*, 1925, vii, 1.

[10] Ledingham, *J. State Med.*, 1926, xxxiv, 1925.

[11] Calmette and Guerin, *Ann. Inst. Pasteur*, 1901, 15.

[12] Camus, *Bull. Acad. de Med.*, 1923, xc, 79.

[13] Walthard, *Schweitzer Med. Wochschr.*, 1926, 854.

litis. Later only direct intracerebral injection was used. Testicles and brains for passage were ground up with sand and a 20% suspension in saline made and centrifuged to remove coarse tissue particles. The presence of virus was tested for by intradermal injection.

The strain of virus used when injected by either method did not produce in any animal a fatal encephalitis or even any symptoms, which could not be ascribed to the mechanical effect of the injection. The virus was found to survive at least 4 days in the brain after intracerebral injection but only for 24 hours after ventricular injection.

With ventricular injection and previous meningeal irritation by sterile broth attempts to adapt the virus to the brain by 24-hour brain to brain passage were negative. Virus present in the first passage brain had completely died out by the fourth passage and further passage did not revive it. Animals of the series allowed to live showed absolutely no symptoms. Brains of 2 animals, dying after ventricular injection, which contained some virus, produced no symptoms when injected into other animals.

With direct intracerebral injection attempts to adapt the virus by brain to brain passage at 4-day intervals were negative. Alternating brain and testicular passage, as used originally by Levaditi, was also without result. The virus could be kept alive apparently indefinitely by alternate brain to testicle transfer but no evidence of any acquirement of intracerebral pathogenicity could be detected and if the testicular passages were omitted the virus soon died out.

In conclusion the strain of virus used is definitely not encephalitogenic for rabbits; if it can be made to produce encephalitis it can be made to do so only with extreme difficulty. In view of the apparent ease with which many European workers have succeeded in producing encephalitis with an ordinary strain of vaccine virus and the lack of positive or negative reports of any such attempts in this country this failure to obtain a vaccine encephalitis in rabbits is considered of interest. An explanation which offers itself is that the power to produce encephalitis depends upon the strain of virus—some strains producing it readily, some only after adaptation and some not at all. The theory of Camus that a virus which causes encephalitis is contaminated by some unknown virus must also be held in mind.

4398

A Study of Acute Infection of the Respiratory Tract in the Ape.

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During an investigation of the type of acute infections of the human upper respiratory tract usually grouped under the term "Common Cold", we have considered the possibility of finding an animal susceptible to infections of this nature. Such animal diseases as distemper in dogs and snuffles in rabbits, though having a certain resemblance to the human disease, seemed in all probability to be too dissimilar from an etiological standpoint to be of immediate value. Our search led us to consider the advisability of employing the anthropoid ape for the purpose. On questioning curators of zoological collections and others having to do with the importation and study of the higher apes, it quickly became obvious that those animals not only frequently suffered from "Common Colds", but that the source of infection was a human being similarly afflicted. The description of the condition bears a striking resemblance to the human disease and not infrequently it is followed by a secondary pneumonia of severe character.

Having assured ourselves of the probable susceptibility of these animals to the condition under investigation, the decision was made to collect a small colony of anthropoids and to study in them the spontaneous occurrence of upper respiratory infection and the nature of the experimentally produced disease. The animals were obtained from dealers, had been exposed to intimate human contact for from 3 months to a year, and some were known to have suffered from acute upper respiratory infection of the type described. Young animals of about 3 years of age and under 30 pounds weight were chosen because of the ease with which they could be handled. The animals were at all times protected from frequent human contact and during the period of experimental observation were under strict quarantine, all attendants employing surgical aseptic technic of sterile hood, mask, gown, and rubber gloves.

The first undertaking was a study of the upper respiratory flora during periods of normal health. This was found to resemble, in respect to organisms present, that of the human upper respiratory tract to a surprising degree. The organisms more or less continuously present are shown in the following table:

TABLE I.
Comparison of percentage incidence of bacteria in noses and throats of normal simians and humans.[1]

		Organisms												
		Gram-negative cocci	Streptococcus non-hemolytic	Bacillus "X"	*B. pfeifferi*	Diphtheroids	Large Gram-positive cocci	*Staphylococcus albus*	*Streptococcus hemolytic*	*Staphylococcus aureus*	Pneumococcus	*B. coli*	Strepto-Bacillus	Total Cultures
Nose	Monkey	17	60	0.6	13	21	8	95	1.2	26	1.2	4	15	169
	Man	1	7	0	0	79	0	92	0.4	36	0.	0	0	265
Throat	Monkey	99	99	21	92	11	5	31	45.	9	0.6	13	0	159
	Man	99	99	49	47	45	45	40	17.	14	2.0	0	0	265

[1] Shibley, Hanger, and Dochez, *J. Exp. Med.*, 1926, xliii, 415.

During the period of observation a number of the animals, while not under quarantine, have contracted upper respiratory infections presumably from chance human contacts. The following symptomatology was the one generally observed: the first sign of upper respiratory infection was a discharge of mucus from both nostrils accompanied by occasional attacks of sneezing. During the act of sneezing considerable amounts of mucus were blown from the nostrils. Somewhat later the nares became obstructed and the breathing audible. In from 3 to 5 days a cough frequently developed, lasting sometimes as long as 2 weeks. In some instances the mucus discharge became purulent and continued from one or the other nostril for as long as 3 weeks. There was some lassitude and loss of appetite and occasionally diarrhea. The temperature was seldom elevated and then only to an insignificant degree. In 3 animals the attack was complicated by bronchopneumonia with marked prostration, loss of appetite, and fever. One of these animals died and the lungs showed pneumonic lesions of somewhat unusual character.

One of the striking features of the picture is the marked increase in numbers and spread in area involved by the potentially pathogenic bacteria already present in the upper respiratory tract.

One of the objects of this investigation has been to determine whether it is possible to communicate to anthropoids by means of a filterable agent a respiratory infection comparable to the human

cold. For this purpose single animals were chosen at a time when they had been free from respiratory symptoms for at least 6 weeks. They were placed in a special room in individual cages and strict quarantine maintained by the technic described. They were kept under observation for from 1 to 2 weeks to test the efficiency of the isolation. No animal under such conditions acquired a spontaneous respiratory infection. A daily study of the bacteriology of the upper respiratory tract was conducted.

As a source of the infectious agent, human beings were chosen with typical manifestations of a common cold of at least moderate severity. Nasopharyngeal washings were obtained within the first 24 hours of symptoms. This material was rapidly passed through a Berkefeld filter V. Both the unfiltered and the filtered washings were carefully studied bacteriologically. To test for the presence of herpes virus, intra-cerebral inoculations into rabbits were made with the filtrate. Usually within an hour from the time the washing took place, from 1 to 2 cc. were instilled into each nostril of the experi-

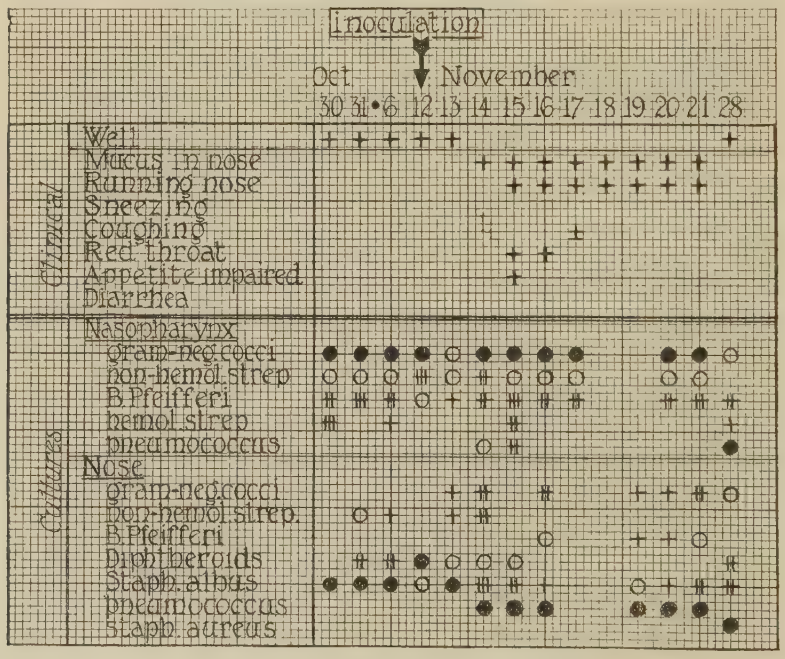

FIG. 1.

Chart showing development of symptoms in experimental animal following inoculation with filtered nasal washings obtained from an early cold in a human. The bacterial flora in the nose and throat before, during and after, is shown. Solid circle indicates organisms present in greatest numbers, blank circle the next most numerous, and plus signs indicate the remaining ones.

mental animal. A second instillation of the same material was made 6 hours later. At first plain broth and heated filtrate controls were carried out, but were later discontinued as inadequate. A different type of control is planned at a period when human colds are not prevalent.

Of the first 7 animals inoculated intra-nasally according to the technic described above, 3 contracted upper respiratory infections similar in their manifestations to the spontaneous infections of the simian and to the "common cold" in human beings. In 2 experiments the symptomatology was ambiguous and in 2 no symptoms developed.

The experiment charted below is a typical example of the phenomena observed. The incubation period of the experimental infection was about 36 hours. The symptomatology and alteration of the bacteriological flora of the upper respiratory tract are shown in the chart. In the experiment charted, a healthy chimpanzee kept in close contact with the experimental animal, contracted, presumably by contact, a spontaneous cold on the fourth day of the experimental infection.

The nature of the active filterable agent is as yet unknown. In one positive experiment the filtrate contained herpes virus, and from the filtrate in all positive experiments a Gram negative anaerobic bacillus of the type described by Olitsky and Gates was cultivated.

4399

Oral Immunization Experiments with Disrupted, Dissolved and Avirulent Pneumococci. Time of Appearance of Immunity.*

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Earlier experiments[1] have demonstrated that when dead pneumococci are fed to white rats, the animals become immune to an injection of virulent germs of the same type. The present report deals with the observations made when mechanically disrupted, bile dissolved and avirulent organisms are fed. It also includes a report of experiments in which rats were fed the pneumococcus grown in

* The author wishes to express his thanks to Mrs. K. L. Harriman for her kindness in providing financial support for the work. He is also indebted to Dr. Wm. H. Park and Dr. Wm. G. Lyle for aid, advice and facilities.

[1] Ross, Victor, *J. Lab. and Clin. Med.*, 1927, xii, 566.

whole milk.† The mechanically opened cells and the bile-dissolved ones were fed in order to learn whether the intact cell is necessary in this mode of conferring immunity, or whether the contents alone will serve as well. In one of the latter experiments some of the animals were examined a few days following the first feeding, instead of much later as was the custom in the earlier part of this work. The purpose was to observe how quickly the protection is created. Feeding with avirulent pneumococcus was carried out with a group of rats to determine whether virulence is an essential factor. Type I pneumococcus was used throughout. The resistance of the rats was tested by injecting the organism intraperitoneally in a volume of 0.20 cc.

Effect of Feeding Mechanically Disrupted Germs. The organisms were grown in glucose meat extract broth, centrifuged and desiccated in partial vacuum. The scaly material was then pulverized in a pebble mill for about 18 hours. Microscopic examination failed to detect any intact pneumococci. Each rat received the equivalent of 50 cc. of culture per day for 16 days. The powdered material was mixed with milk and placed in the cage. The animals were well protected by this method, surviving 1000 to 10,000 fatal doses. Doubtless, some of the germs escaped disruption in the mill, but it is believed that these were too few in number to have been responsible for the pronounced effect observed.

Effect of Feeding Organisms Grown in Milk. Sterile milk was inoculated with virulent broth cultures of pneumococcus and incubated for 18 hours. Several methods for killing the germs were tried. Heating at 60° C. for one hour was used. This causes separation and curdling. After shaking thoroughly, the milk was placed in the cage. The average amount consumed by each rat was 3 cc. per day. Feeding took place from 11 to 17 days. The rats were protected against 100 fatal doses, in one instance against 1000 fatal doses. The experiment was repeated using a group of rats which were older. The younger rats appear to give better results, an observation made also when bile dissolved cells were employed. This difference was not observed when the HCl killed whole cell was used.[1] Although it requires confirmation, it seems that better immunity was obtained when 3 cc. pneumococcus milk culture is fed than when the organisms from the same quantity of

† It has previously been shown that pneumococci grown in broth, centrifuged and suspended in milk give excellent results. I am indebted to Dr. Krumwiede for the suggestion to grow the organisms directly in milk, thus avoiding the need for centrifuging.

broth culture are used. Heating at 60° C. for one hour does not apparently destroy the antigen. It has previously been shown that heating at 80° C. for 2 hours is destructive.[2]

Effect of Feeding Avirulent Pneumococcus. The organism was obtained by treating a glucose meat extract culture with HCl acid, and subculturing before growth was killed. The organism which was quite virulent before, became avirulent for mice, failing to kill when injected intraperitoneally in 1 cc. quantities. It was bile insoluble and showed slight and equal agglutination in the 3 fixed types of antisera. No soluble specific substance was formed. The avirulent cultures were killed by 2 hours' contact with N/15 HCl acid and the centrifuged germs mixed with cracker meal. Nine daily feedings of the pneumococci from 50 cc. culture were given each rat. No immunity follows the administration of the avirulent germ.

Feeding of Bile Dissolved Pneumococcus. Two experiments were done in which the pneumococcus was grown in beef heart broth, and after being centrifuged, was dissolved in sodium glycocholate. About 100 mgm. of the bile salt were used for a litre of growth. After remaining at room temperature for about 2 hours, microscopic examination generally showed the presence of an occasional intact cell. This material which was prepared fresh daily, was mixed with cracker meal and fed to the rats on 21 days in quantities equivalent to 50 cc. culture per rat per day in the first experiment, and the equivalent of 25 cc. culture per rat per day for 6 days in the second. The latter gave a better immunity, perhaps because the animals used were younger. Some of the rats were resistant to 10,000 fatal doses. When the Berkefeld (N) filtrate of sodium glycocholate dissolved pneumococci was administered to rats by mouth in quantities equivalent to 30 cc. culture per rat per day for 3 days protection was definitely present 3 days after the first feeding. Two experiments were done to determine whether the immunity appears sooner. It was found that 48 hours after a single feeding of Berkefeld filtrate of sodium glycocholate dissolved pneumococci the animals survived an injection of at least 1000 fatal doses. The experiments in which mechanically opened, bile dissolved and milk grown pneumococci were used, were also carried out on mice. As was the case following the use of HCl acid killed germs, the results obtained with these forms were comparatively poor. An effort is being made to learn why there is this difference between rats and mice.

[2] Ross, Victor, *J. Immunol.*, 1926, xii, 237.

4400

The Chemistry of the Estrus Producing Hormone.

CASIMIR FUNK.

From the Research Laboratory of Gaston Grémy, Paris.

The publications of Allen and Doisy in which estrin (folliculin) has been shown to be present in the follicular fluid of castrated mice,[1] have been followed by publications from other authors who detected this hormone in the ovary and, more particularly, in the placenta. Loewi and his coworkers have since shown the hormone to be present in the urine of pregnant women,[2] and Doisy and Veler have devised a convenient method for extracting it.[3]

Little seems to be known of the chemistry of this hormone. Earlier authors considered estrin to be a lipoidal substance present in the non-saponifiable fraction (sterol fraction). Somewhat later several investigators (particularly Zondek[4]) prepared the substance in a water-soluble form. No detailed description of the method used in preparing the substance in water-soluble form seems to have been given; and it seems somewhat difficult to understand how a substance first described as lipoidal in character can be rendered entirely water-soluble by an extraction process.

Some observations made by the writer during the past year may throw light on the matter. It has been found that estrin is very sparingly soluble even in hot water, and that most of the hormone occurs in the free state in the ovary, placenta or urine. Saponification is not necessary. The reason why the hormone has been supposed to reside in the unsaponifiable (cholesterol) fraction is because it forms salts with strong alkalies which are fairly well soluble in ether. Estrin forms soluble sodium, potassium, ammonium, and barium salts. Making use of the property just described, estrin fractions weighing 0.01 mg. per rat unit are easily obtainable. Such preparations show an acidity equivalent to 43.2 cc. of N/10 sodium hydroxide per 100 mg. of substance. The similarity in chemical behavior of estrin and phenols renders further purification not easy

Using urine as starting material, one often encounters further difficulty, due to the frequent use of phenolphthalein as a laxative,

[1] Allen and Doisy, *Am. J. Anat.*, 1924, xxxiv, 133.

[2] Loewi and Lange, *Klin. Wochenschr.*, 1926, v, 1038; Loewi and Voss, *Ibid.*, 1928, vii, 1376.

[3] Doisy and Veler, PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 806.

[4] Zondek, *Klin. Wochenschr.*, 1928, vii, 485.

and, therefore, to its presence in mixed urines. It is very difficult to separate the hormone from the phenolphthalein.

Estrin appears to be a phenol or an alcohol forming easily soluble salts with strong alkalies.

Aqueous solutions of estrin appear to be slightly less active (as measured on castrated rats) than solutions in oil of equal strength. This might be due to a more rapid elimination of the aqueous extract.

4401

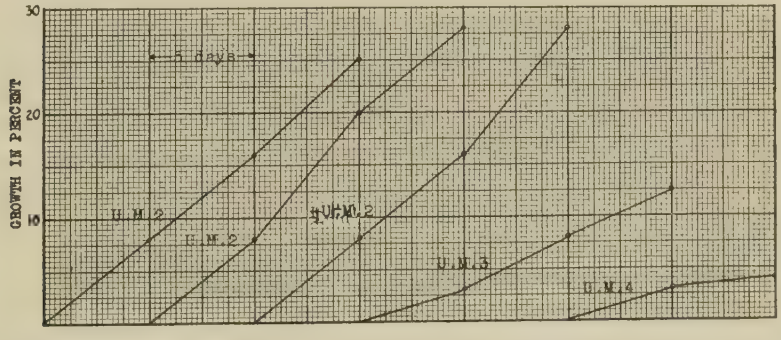
The Male Hormone. II.

CASIMIR FUNK, BENJAMIN HARROW AND A. LEJWA.

*From the Research Laboratory, Gaston Grémy, Paris, and the Department of Chemistry, College of the City of New York.**

In a previous communication[1] we reported that the male urine contains appreciable quantities of the male hormone, as evidenced by the increased size of comb in castrated cocks.

In the course of the work we noticed that the male hormone (testiculin) shows chemical properties similar to those of estrin. For example, testiculin, like estrin, can be extracted from urine by means of chloroform. The urine to be used is adjusted to a pH of 4.0-4.5 and continuously extracted with chloroform for 6 hours. The separated chloroform layer is evaporated and the residue dissolved in an appropriate amount of olive oil.



Action of the daily injection of chloroform extracts (representing 100 cc. urine) on groups of six castrated cocks.

* This work has been made possible through grants obtained from Mr. A. Heckscher, Mr. Harold McCormick and Mr. Jules S. Bache.

[1] Funk and Harrow, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 325.

Using urinary extracts, we find that the comb growth is proportional to the amount of hormone present (see chart). We have selected as our cock unit that amount of testiculin which, when injected daily into each of 6 castrated cocks, will give an average increase in comb of 10 mm. in 10 days. Such a cock unit is found in about 75 cc. of an active urine.

The administration of chloroform extracts has permitted the use of more concentrated preparations, without the toxic effects of the urine. Changes in size of comb, in turgor and color are plainly noticeable after a few days of treatment.

As a preliminary to the study of the functional test of gland activity, samples of mixed urines were extracted with chloroform, the residues dissolved in oil and each sample injected into six well-castrated cocks.

Urine sample U.M.2, was obtained from men in the prime of life. The curves show how unifom is the effect of this extract on 3 different groups of animals. U.M.3 represents the urine from men of various ages, and U.M.4 from men between 50-70 years of age. The variation in hormone content is quite evident.

It remains to be seen whether the differences observed can be of service in diagnosis.

4402

Experimental Treatment of Cardiac Arrhythmias.

LINN J. BOYD, J. G. BRODY AND D. ANCHEL.
(Introduced by Israel S. Kleiner.)

From the Department of Pharmacology and Experimental Medicine of the New York Homeopathic Medical College and Flower Hospital.

Since weakening of the heart muscle leads to incomplete emptying and progressive dilatation, which in turn results in impaired conduction, arrhythmias, and auricular fibrillation, it is conceivable that these conditions would be improved by increasing the force of the heart and slowing its rate by digitalis, or by relieving its load.

In order to accomplish the latter we have bled 5-10 cc. per kg. or changed the distribution of the blood in the active circulation by snake venom *(Lachesis lanceolatus),* histamine, peptone and nitrites.* These drugs were injected into the femoral vein of cats pre-

* We used 47 animals, of which 10 were controls. 34 gave positive and 3 negative results, because of dosage. In many experiments the procedure was repeated.

viously anesthetized with urethane. Respiration and blood pressure curves were taken and checked up by simultaneous electrocardiograms.

After a normal tracing and electrocardiogram were taken, cardiac arrhythmias were produced by chloral or aconitine, mostly the former because of its more uniform results. Fifty mg. of chloral hydrate per kg. was the initial dose used. Occasionally this was sufficient to cause an irregular heart, but as a rule it had to be repeated 2 or 3 times. As soon as the tracing showed an irregularity, an electrocardiogram was taken, and then the therapeutic drug was injected. The electrocardiograms before the injection revealed numerous right and left ventricular extra-systoles, auricular extra-systoles, nodal rhythm, or partial heart block. Some of these electrocardiograms showed a single irregularity, while others showed combinations of several irregularities. In untreated animals the arrhythmia lasted for 15 to 45 minutes. A few animals died within 15 minutes, the irregularity persisting till death.

The first drug used was *Lachesis lanceolatus,* 0.025 mg. per kg., and, as a rule, in about one minute the heart became regular, as shown by the tracing and the electrocardiogram.

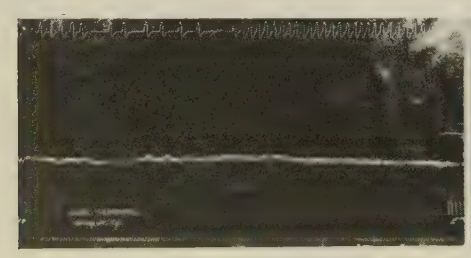


FIG. 1.

The irregular chloral heart became normal in about 1 minute after the injection of Lachesis. Note the effect on respiration.

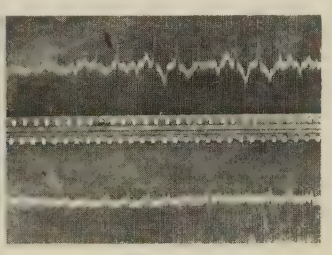


FIG. 2.

The upper electrocardiogram was taken just before Lachesis was injected. It shows a number of ventricular extrasystoles. The lower electrocardiogram which was taken at the point marked E10, at the extreme right of the previous tracing, shows a normal sinus rhythm.

Histamine 0.03 mg. per kg., peptone 100 mg. per kg., nitroglycerine 0.4 mg. per kg., withdrawal of about 10 cc. of blood per kg., or pressure on the Inferior Vena Cava gave similar results. The reinjection of the blood withdrawn, or the injection of saline in slightly larger quantities than the amount of blood withdrawn caused the immediate return of the cardiac irregularity.

The fact that bleeding and drugs causing a fall of blood pressure

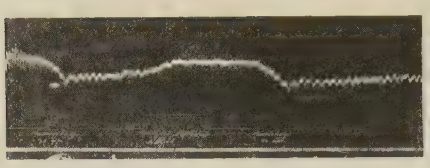


FIG. 3.

Note that the irregular chloral heart returns to normal after bleeding (20 cc.), that the irregularity returns when the blood is reinjected, and becomes regular again when 20 cc. of blood is once more withdrawn.

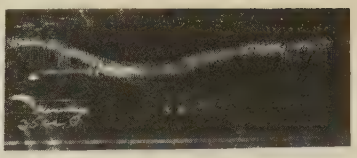


FIG. 4.

The irregular chloral heart becomes regular when 35 cc. of blood is withdrawn and becomes irregular again when 45 cc. of saline is injected.

remove the arrhythmias in a weakened heart, and that the reinjection of rather small amounts of normal saline precipitates the irregularity, indicate the importance of relieving the excessive load in cardiac disease.

4403

Addiction Edema and Withdrawal Edema in Morphinized Rats.

S. H. FLOWERS, E. S. DUNHAM AND H. G. BARBOUR.

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Healthy adult male rats on a diet of whole wheat (plus 2% NaCl) were subjected to morphine addiction starting with daily doses of 10 mgm. morphine sulphate (5% solution) per 100 gm. rat, increased every 3 or 4 days up to 100 or more mgm. per kilo. The rats ate well throughout the work except in the early stages of morphine addiction. Weight was usually well maintained. The rats were killed in pairs at various stages, and samples of brain, muscle, skin and liver were dried to a constant weight, with the results shown in the table.

Water Content of Tissues. In the first series the rats were first killed on the 28th day of addiction when all of the tissues were found abnormally wet. The relation of the solids to normal was: brain –22%, muscle –9%, skin –22%, liver –13%. On withdrawal the only analyses in this series were made on the fourth day when the brain was found normal and the other 3 tissues about half recovered. In the second series the changes were followed every 3 or 4 days, and withdrawal begun on the twenty-fourth day.

Addiction edema was found at its maximum at the following

times; in the brain, tenth day; muscle, twenty-third day; skin, third and fourteenth days; liver, tenth day. On the last (twenty-third) day of addiction only the muscle showed maximal edema, liver and skin were half recovered and brain nearly normal. Although never much above the normal level, the brain solids usually varied inversely with those of the skin.

The first day of withdrawal was marked by the *sudden onset of a new edema* in the brain and liver, compensated by drying of the skin. The brain solids change in the one day amounted to −14%, the skin solids *gaining* 10%. The third day of withdrawal shows results all agreeing closely with the fourth day (only day studied) of the first series. The water changes in the tissues may be summarized as follows:

	ADDICTION	WITHDRAWAL
Brain	wet gradually; late recovery	wet suddenly; recovery; relapse
Muscle	wet gradually	recovery; relapse
Skin	wet suddenly; late partial recov.	dry suddenly; shift to wet
Liver	wet gradually; partial recovery	wet suddenly; partial recovery

Thus we have an *addiction edema* perhaps due largely to malnutrition (from digestive impairment?) and a *withdrawal edema* in brain and liver corresponding to the withdrawal edema demonstrated in dogs,[1] and compensated by drying of the skin. The withdrawal edema occurs suddenly and is more temporary in rats than in dogs. It appears to be parallel to the following changes: on the first day of withdrawal were found soft stools, a sudden marked fall in body weight and body temperature (in both series) all of which conditions were recovered from gradually in the succeeding few days.

The food and water intake and urine volume were followed in 7 rats of the second series during the last 6 days of addiction and for a like period during withdrawal.

Addicted rats consumed normal amounts of food. Withdrawal produced an immediate increase to a level about 25% above normal.

Morphine addiction increased the water intake to 25 to 50% above normal and the urine by at least 200%. On the first day of withdrawal the water intake fell to *below normal* but by the fourth day had increased to *double the addiction level.* The urine volume varied similarly but not so greatly.

Supernormal water drinking during addiction followed by subnormal intake immediately on withdrawal is in striking contradic-

[1] Barbour, H. G., Hunter, L. G., and Richey, C. H., *J. Pharm. and Exp. Therap.*, 1929, in press.

tion to a uniform experience with dogs in which morphinism suppresses the water exchange.

Conclusions: Morphinism in rats causes edema which tends to advance and decline at different rates in different tissues. Early and extensively affected is the skin. The water exchange becomes decidedly increased. Sudden withdrawal induces a fresh edema, especially of the brain, the skin passing first through a dry stage. The water exchange is first depressed, then greatly increased. Ingestion of food has not varied sufficiently in amount to account for any of the findings.

4404

Further Notes on the Relation of Nutrition to the Development of Cancer.

MONTROSE T. BURROWS.

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In previous papers[1] we had shown that malignant tissue differs from the normal tissues of the body in that it contains no demonstrable or very little of the growth promoting fat soluble vitamins. We had also shown that many of the so-called cancer producing substances and forces, such as coal tar, other lipoid solvents, x-rays, radium, other lights and heat act directly to dissolve or otherwise remove these fat soluble vitamins from the tissues. While these studies were interesting in that they indicated that cancer may be nothing more than the result of the removal of the fat soluble vitamins from a tissue, it seemed quite unlikely that a lipoid solvent, no matter how often applied to a tissue, could remove sufficient of the vitamin to induce cancer. When a solvent is brought into contact with a medium it removes only a certain fraction of the dissolved substances from the medium.

That the local action of coal tar is not alone responsible for coal tar cancer has been indicated further by the fact that all animals painted with coal tar do not develop the disease while cancers have been known to develop in man years after a single injection of paraffin or a single dose of x-rays. There seems also to be little evidence to show that the appearance of cancer in man is related directly to the extent or severity of a previously existing local lesion. In fact, many patients carry definite precancerous lesions for years without

[1] Burrows, M. T., and Jorstad, L. H., *Am. J. Physiol.*, 1926, lxxvii, 24.

any evidence of malignant degeneration. All these facts indicate, therefore, that factors other than the local lesion must be responsible for the malignant changes. To throw light on the possible nature of these other factors it has become of interest to study more carefully the action of coal tar and x-rays on animals fed a normal dietary and animals fed varying quantities of both vitamin B and the fat soluble vitamins. These studies showed that the typical precancerous change can be induced more readily by a single application of coal tar or x-ray in a rat fed a diet either high in vitamin B or low in the fat soluble vitamin than by repeated applications in the well nourished rat. We also found that these applications of coal tar or other lipoid solvents cause not only local changes in the tissue but a drop in the fat soluble vitamin content of the whole organism. The appearance of more cancers in animals after repeated applications of coal tar may be due, therefore, as much to the systemic effect of the solvent as to the local effect.[2]

In other experiments with growing cells *in vitro,* facts were gleaned to show that the source of the protoplasmic lipoids of body cells is probably the fat soluble vitamins of the food. We were unable to find any evidence to show that body cells can grow unless supplied with these vitamins. It is the excess of these vitamins in the tissue about the cells or in the yolk of the egg which inhibits the growth of the cells. It became evident, therefore, that any tissue stimulated to grow in a medium free from the fat soluble vitamins but containing ample of other nutrient materials might use up its excess of these vitamins and thus transform itself into a cancerous tissue. Berkefeld filtrates of the Jensen sarcoma of rats contain large quantities of growth stimulating substance but no demonstrable quantities of the fat soluble vitamins. Tests of these filtrates in feeding experiments and in the tissue culture have given no evidence that they contain other specific or toxic substances. It became of interest, therefore, to use these filtrates to stimulate the growth of the tissue. It seemed evident if we could find a normal tissue with the proper nutrient reserve it would become changed readily by them into a cancer.

The storage of food in tissues varies not only with the animal but also with the age of the animal. Fragments of the tissues of embryos of various ages and of adults were treated with Berkefeld filtrate of this sarcoma and then transplanted under the skin of young rats. A few malignant sarcomas developed in the fragments of 15 and 16 day rat embryos.[3]

[2] Jorstad, L. H., *J. Cancer Research,* 1926, x, 229.

[3] Burrows, M. T., *Arch. Path. and Lab. Med.,* 1927, iv, 168.

The question arose, therefore, may not the lesions produced by coal tar, x-rays, etc., be only precancerous lesions. The cancer may be the result of a later decrease in the normal nutrition of the animal. In proof for this belief we had already reported a case in 1927.[4] The case was that of a young woman of my acquaintance who placed herself on a diet consisting of chopped meat and vegetables which she boiled twice, pouring the water off after each boiling. She ate little or nothing more than this diet for a period of 3 weeks. Eleven years previously she had received an injection of camphor in oil into the subcutaneous tissue of her right arm. During the time she took the abnormal diet the tumor on her arm became active, reaching the size of a walnut, only to recede again in 7 days on a normal diet rich in vitamins. We removed the tumor later, finding it to be a typical camphor oil tumor.

To throw further light on this problem it had become of interest to attempt to prepare solutions of homologous vitamin B obtained from sources other than tumor tissue, and to study carefully the history of cancer cases to see if a drop in nutrition preceded the development of the cancers. This latter study has been most interesting because it indicates not only that a drop in nutrition precedes the development of all the cases of cancer studied so far but it gives also for the first time some understanding of the age factor in cancer and how many infections, as well as hereditary and acquired defects, may affect nutrition. According to our interpretation of the above experiments it was not only the presence of the growth stimulus which led to the development of sarcomas in the embryonic tissue but to the presence of the proper amounts of other nutrient material in the tissue and the medium.

Forty-three cases of cancer have now been studied. These cases have varied between the ages of 23 and 75 years. All of them have had definite precancerous lesions of one form or the other. In eight of the breast cases the precancerous lesion developed with a recent or earlier existing cervicitis. In four breast cases the precancerous lesion dated back to inflammation acquired during the nursing of children. Four cases of cancers of the cervix could be traced to lacerations. Bruises localized 2 cancers of the face. In the other cases long standing chronic pyogenic infections, injuries from the use of tobacco, alcohol, or other cancer producing substances were evidently the localizing causes of the malignancy.

In none of these cases did the cancer appear, however, before

[4] Burrows, M. T., and Jorstad, L. H., *J. Am. Med. Assn.*, 1927, lxxxviii, 1460.

there was definite evidence of a drop in the general nutrition of the patient. In 8 of the cases deliberate or starvation from poverty preceded the development of the cancers by 1 to 25 years. The cancer developed in 8 cases with the recurrence of an active tertiary syphilis. Starvation from worry associated with gastric disturbance lasting over 2 years preceded the development of the cancers in 2 cases. Poor dietary in the treatment of diabetes preceded the development of the cancer in another case. Chronic prostititis with arthritis and chronic tonsilitis with arthritis and a kidney lesion were present in 2 cases. In the other 22 cases abscessed teeth preceded the development of cancers.

A few of the earlier cases had suffered recurrence of their cancers after careful operations. Removal of the focal infections together with the cancers and a good dietary has been followed by no recurrences for a period of 10 months in 4 of these cases. Where the general condition of the patient could not be improved in 2 of the earlier cases the cancers recurred quickly after most careful operations and x-ray treatment.

4405

Duration of Sleep and the Insensible Perspiration.*

W. R. MILES.

From the Psychology Department, Stanford University.

Sanctorius[1] has an aphorism, No. XV in his Sect. IV on Sleep and Vigilance, which reads as follows: "If the night's rest be less than usual, there is a diminution in the exhalation of the concocted perspirable matter, but the perspiration of crudities is augmented." On the first point I have seen no actual data. Benedict and Root[2] state that the insensible loss is less with quiet sleeping and more with restlessness. A hot uncomfortable night increases the weight loss per hour. These authors give a good review of the literature on the subject of insensible perspiration. The papers like the work of

* The equipment for this study was made possible by the Thomas Welton Stanford Fund for Psychological Research.

1 Sinclair, "Code of Health and Longevity," Edinburgh, 1807, vol. iii, p. 167.

2 Benedict and Root, "Insensible perspiration: Its relation to human physiology and pathology," *Arch. Int. Med.*, 1926, xxxviii, 21; also Benedict and Joslin, "A Study of Metabolism in Severe Diabetes," Carnegie Inst. Pub. No. 176, Washington, 1912, p. 91.

Sanctorius in 1614 have mostly been self-studies. Dr. F. G. Benedict has done more than anyone else or probably than all others combined to point out the importance of research in this field and to set the facts in array.

The data given herewith represent self-observation and cover 40 nights, nearly consecutive, beginning April 24th and ending June 24, 1928. So far as climatic conditions are concerned the data are representative for Stanford University, California. During this period the relative humidity of outdoor air at night ranged from 90 to 100% while in the middle of the day it was usually around 30%. The mean noon day temperature for April, May and June in this locality was 63°, 68° and 73° F. respectively. The night temperature in May for example is close to 53°, dropping about one degree for every 2 hours as follows:

May 10 P. M.	12 P. M.	2 A. M.	4 A. M.	6 A. M.	8 A. M.	N
55°	54°	53°	52°	51°	58°	68°[3]

The sleeping was indoors with windows wide open The nude weights were taken on platform scales ("silk scales") such as are recommended by Benedict. Our scales were provided with a pillar 36 inches high which made self weighing quite convenient and accurate. The beam was graduated in 5 gm. divisions but the scales had a sensitivity of 10 gm. The data are given in Table I. In place of giving the time in bed as hours and minutes, it is recorded in the table as hours and decimals of hours. The range is from 5.33 to 8.75 with an average of 7.31, A. D. .63. Therefore the coefficient of variability for the time spent in bed, which is practically equal to the time between weighings, is 8.7%. The total weight loss recorded for the different nights ranged from 195 to 395 with an average of 288 gm. The A.D. is 47 gm. which amounts to 16.3% of the mean. The product-moments correlation between time in bed and loss in weight was .81 ± .03. The column giving the derived values of loss per hour exhibits a range from 29.0 to 49.0 gm. The average is 39.1 gm., A.D. 4.1 gm. C. of V. 10.5%. The gross body weight for this subject was 78 kilos and during the progress of the experiment there was a gradual change of body weight from 79 kilos on April 24th to 77 kilos on June 24th. The subject was working rather strenuously throughout this period which represents the spring quarter of the academic year, but there was no routine that absolutely forced him to rise at a certain time in the morning. The longer sleeping periods were

[3] Data supplied by Professor J. G. Brown, Physics Department, Stanford University.

TABLE I.

Weight loss by insensible perspiration during sleep. Observations on W. R. M. for 40 nights. Net body-weight 78 kg., height 178 cm.

Month	Night	Time in bed	Loss in weight	Loss per hour	Average for 5 day periods
1928		hrs.	gm.	gm.	gm.
April	24-25	6.90	235	34.0	
,,	25-26	8.17	320	39.2	
,,	26-27	8.08	350	43.3	
,,	27-28	6.92	280	40.5	
,,	28-29	8.50	380	44.7	40.6
,,	29-30	7.25	295	40.7	
May	1-2	8.25	380	46.1	
,,	2-3	7.42	290	39.0	
,,	3-4	7.83	385	49.2	
,,	4-5	6.00	210	35.0	42.4
,,	5-6	5.33	220	41.3	
,,	6-7	6.25	195	31.2	
,,	7-8	7.83	280	35.8	
,,	8-9	5.92	245	41.4	
,,	10-11	7.58	330	43.6	38.6
,,	11-12	7.25	275	38.0	
,,	12-13	8.00	335	41.9	
,,	13-14	7.00	250	35.7	
,,	14-15	7.00	310	44.4	
,,	15-16	7.75	290	37.4	39.4
,,	16-17	8.50	300	35.3	
,,	17-18	7.50	315	42.0	
,,	18-19	7.92	250	31.6	
,,	19-20	8.00	350	43.7	
,,	20-21	8.75	360	41.1	38.7
,,	21-22	7.00	255	36.4	
,,	22-23	6.92	325	47.0	
,,	23-24	7.00	290	41.5	
,,	24-25	7.08	205	29.0	
,,	26-27	8.17	375	46.0	39.8
,,	27-28	7.25	215	29.7	
,,	28-29	7.25	245	33.8	
,,	29-30	6.33	225	35.5	
,,	30-31	6.50	260	37.7	
June	31-1	7.25	285	39.4	35.6
,,	1-2	7.83	320	40.9	
,,	2-3	6.00	200	33.4	
,,	3-4	7.50	285	38.0	
,,	11-12	8.08	395	49.0	
,,	24-25	6.50	210	32.3	39.2
Ave.		7.31	288	39.1	
A.D.		.63	47	4.1	

sometimes but not always associated with the week-end. In general the length of the rest period varied quite irregularly throughout the entire group of days. It was not varied according to any prearranged plan.

The 8 successive groups of 5 days show mean losses per hour that are not very far different but average a little less in the last half of the period covered. Our total average of 39.1 gm. equals 0.5 gm. in-

sensible loss per kilo body weight per hour. The data cited by Benedict and Root as well as their own data show about this value except that for their heavier subjects, above 70 kg., it tends to be less. At present we cannot say whether our greater loss than their heavier subjects is climatic or individual difference.

If we classify our data in reference to duration of time in bed, which is here about synonymous with duration of sleep, we find the following:

8 nights of 6.5 hrs. or less, ave. loss per hr.----------36.2 gm.
16 nights of 6.55-7.5 hrs., ave. loss per hr. ----------38.0 gm.
16 nights of 7.55 hrs. or more, ave. loss per hr.---------41.8 gm.

The differences are consistent but they are not large and fail of statistical significance. However, they tend to verify Sanctorius' aphorism quoted above and probably indicate that with the sounder or fore part of the sleep period there is a slower loss. In the longer normal sleep the latter portion approaches more to the waking condition and has a higher rate of loss.

4406

Classification of "Fibroblasts" According to Their Physiological Properties.

RAYMOND C. PARKER AND ALBERT FISCHER.

From the Kaiser Wilhelm Institute für Biologie, Berlin-Dahlem, Gastabteilung Dr. A. Fischer aus Kopenhagen.

Years ago it was stated that epithelial cells cultivated *in vitro* dedifferentiated or returned to an indifferent cell type which could not be distinguished from fibroblasts.[1] However, the conclusions drawn at that time were mainly due to an inefficient technique which resulted in impure strains of tissue cells. Since we are now able to cultivate pure strains of tissue cells indefinitely, we know that the cells, under the conditions of the experiment, remain typical and retain their morphological characteristics throughout the entire period of cultivation. In the case of fibroblasts and epithelial cells, it has become a relatively simple matter to distinguish between the 2 cell types. One is aided not only by marked morphological differ-

[1] Champy, C., *Compt. rend. Soc. biol.*, 1912, lxxii, 987; Uhlenhuth, E., *J. Exp. Med.*, 1916, xxiv, 689.

ences in the cells themselves, but also by striking differences in the mode of growth of the cell colony.[2]

Within recent years, we have been mainly interested in studying the maintenance of special properties of tissue cells under various experimental conditions *in vitro*. We now know that cell types which are morphologically similar may possess functions which are extremely diverse. Pigment epithelium, when cultivated *in vitro*, continues to produce pigment;[3] thyroid epithelium produces colloid;[4] cancerous epithelium, when inoculated *in vivo*, produces cancer.[5] These findings show clearly that cell properties are maintained under the conditions of cultivation *in vitro*.

The experiments, which are to be reported here, show that various mesenchyme cells, which would all be designated morphologically as "fibroblasts," possess properties and characteristics which are decidedly unlike. Up to the present time, the fibroblasts which have been most intensely studied *in vitro* have been derived from the embryonic chick heart. The purpose of the present experiments was to study one of the more elementary properties of living tissues, namely, their inherent growth potencies. In this connection, heart fibroblasts were compared with other fibroblast-like cells isolated from various tissues of the organism. Four cell strains were isolated simultaneously from the same embryo. The first strain of cells was isolated from the periost of the frontal bone of the skull, according to the method described by Dolschansky.[6] These will be referred to as osteoblasts. The second strain was derived from the perichondrium of the sphenoid. These cells will be referred to as chondrioblasts. The third strain consisted of fibroblasts from the muscle of the leg; the fourth, fibroblasts from the heart. Carrel's 17-year-old fibroblasts were also used for the purpose of comparison.

We know that the activity of a tissue *in vitro* at a given instant is a function of its activity at the preceding instant and of the concentration in the pericellular fluid of the substances which increase or decrease cell activity.[7] Accordingly, the experiments demanded that the different strains be cultivated under absolutely identical conditions from the moment of isolation. This was rigidly carried

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[2] Fischer, A., *J. Exp. Med.*, 1922, xxxv, 367.

[3] Ebeling, A. H., *Compt. rend. Soc. biol.*, 1924, xc, 562.

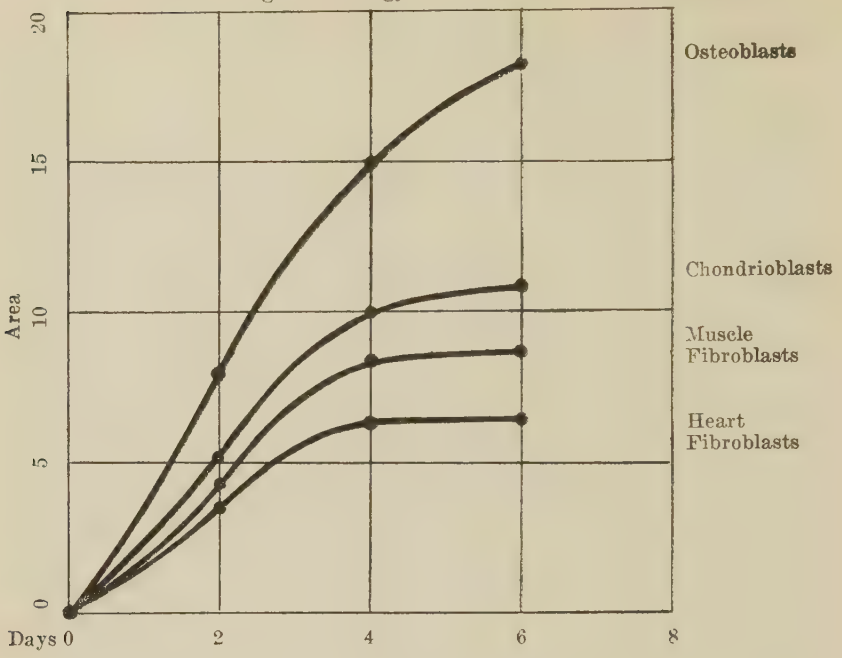
[4] Ebeling, A. H., *Compt. rend. Soc. biol.*, 1924, xc, 1383.

[5] Fischer, A., Demuth, F., Laser, H., und Meyer, H., *Münch. med. Wochenschr.*, 1928, lxxv, 651.

[6] Dolschansky, L., *Z. f. Zellforschung u. mikr. Anatomie*, 1929, viii, 789.

[7] Carrel, A., *Physiol. Rev.*, 1924, iv, 1.

FIG. 1. Residual growth energy of the four strains of fibroblasts.



out. On different occasions, only the zones of new growth were retained in order to equalize the density of the tissue fragments and to eliminate thoroughly all traces of the original explants. It was found that the tissues differed vastly in their rate of proliferation as well as in their response to increasing concentrations of embryonic tissue juice. The comparative experiments were made in Carrel flasks and the rate of growth was measured by the usual method. Reference will be made here to a few typical experiments which well illustrate the general findings. From Fig. 1 it can be seen that the 4 different strains of fibroblasts exhibit different residual growth energies[8] when cultivated in Tyrode solution alone. That for the osteoblasts is highest; next in order come the chondrioblasts, muscle fibroblasts, and last of all, the heart fibroblasts. Carrel and Ebeling have clearly shown that the rate of growth of the heart fibroblasts is a function of the concentration of embryonic tissue juice in the medium.[9] This fact has been substantiated for the heart fibroblasts, and for other types of fibroblasts up to certain concentrations of the growth promoting substances. It appears to be clear that those

[8] Carrel, A., *J. Exp. Med.*, 1923, xxxviii, 521.

[9] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 317.

strains which show the highest residual growth energy cease to grow at a relatively low concentration of embryonic tissue juice.

Accordingly, with these results before us, it remains only to repeat that tissue cells cannot be defined solely according to their morphological characteristics. Their physiological properties should constitute the first claim to any definitions which are to be attempted.

4407

Differentiation and Proliferation in vitro.

ALBERT FISCHER AND RAYMOND C. PARKER.

From the Kaiser Wilhelm Institute für Biologie, Berlin-Dahlem, Gastabteilung Dr. A. Fischer aus Kopenhagen.

In an earlier paper[1] the conditions were described under which undifferentiated sheets of epithelial cells became differentiated into a tissue which formed tubules. When pure strains of epithelial cells and fibroblasts were cultivated together in the same medium, it was found that the 2 cell types retained their individual characteristics indefinitely.[2] The connective tissue cells promptly surrounded the epithelial cells which arranged themselves into structures resembling glandular tissue, with distinct luminae. This same observation was made by Drew[3] in connection with skin epithelium and carcinoma cells. Drew stated that differentiation occurred only as the result of the interaction of 2 different tissues. However, one of us[1] has shown that differentiation is not necessarily dependent upon the interaction of other tissues. In order to obtain keratinization and tubule formation in cultures of pure epithelial cells, it is only necessary to refrain from cutting the culture through the center when transferring it from one medium to another. In other words, differentiation begins to take place as soon as the tissue becomes thick and in such a state that the central portion becomes badly nourished and poorly aërated. When epithelial cultures grow in membrane formation, the growth is rapid and extensive; when the tubular type of colonies appear, growth proceeds very slowly and the actual increase in mass is relatively small, although the outgrowing tubules may become of very considerable length.

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[1] Fischer, A., *J. Exp. Med.*, 1924, xxxix, 585.

[2] Ebeling, A. H., and Fischer, A., *J. Exp. Med.*, 1922, xxxvi, 285.

[3] Drew, A. H., *Brit. J. Exp. Path.*, 1922, iii, 20; *Lancet*, 1923, i, 785, 833.

The experiments, here to be described, deal with factors which, it is believed, play a very considerable rôle in the mechanism of differentiation. The material used consisted of a 6 months old strain of fibroblasts derived from bone, but from which all trace of the original tissue was very early eliminated by selection of only the new outgrowth at the time of transfer. This process was repeated at frequent intervals, yet the cells never lost a very remarkable property, namely, the ability to transform themselves into typical bone cells when this was permitted under the conditions of the experiment. As has already been indicated elsewhere,[4] these fibroblasts possessed no morphological features which rendered them readily distinguishable from the common connective tissue fibroblasts isolated from the embryonic heart.

In order to study carefully the progressive stages in this process, a large series of such fibroblast cultures were isolated from the new growth and subjected to varying amounts of growth-promoting substances. From time to time representative cultures from the different series were fixed, cut in serial sections, and stained for histological examination. The findings will be reported more fully at a later date.

When the usual method of tissue cultivation was employed, *i. e.*, the combination of plasma and embryonic tissue juice, this transformation proceeded very slowly or not at all. But when the rate of multiplication of the cells was suppressed by a reduction of the growth-promoting substances in the medium, differentiation occurred. Cultures which were carried in flasks, or by the hanging-drop method on slides in pure plasma, the center of the culture being retained at each transfer, showed, after a few passages, the formation of typical bone tissue. Such a transformation was distinctly retarded in the control cultures, which were cultivated for the same length of time in equal parts of plasma and embryonic tissue juice.

These experiments have demonstrated that proliferation inhibits the normal function of the cells, namely, the building up of structures peculiar to their type. Hence, reproductive quiescence is an important factor in differentiation.

[4] Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 580.

4408

A New Technique for the Study of Tissues in a State of Latent Life in vitro.

ALBERT FISCHER AND RAYMOND C. PARKER.

From the Kaiser Wilhelm Institute für Biologie, Berlin-Dahlem, Gastabteilung Dr. A. Fischer aus Kopenhagen.

We know that active proliferation prevents the normal functions of tissue cells *in vitro*.[1] For this reason we have found it necessary to modify the present technique of tissue cultivation in order to study more carefully the innate properties of the cells and their relationship one to another. The methods developed by Carrel and his collaborators have provided an excellent means for the study of growth phenomena in general. In the hands of the physiologists, cultures of growing tissues have been mainly used as reagents for the study of the rate of cell proliferation under all possible conditions. But morphologists and embryologists, who expected much from the method, have been rather disappointed. The characteristic structure of the tissue cultivated was very early lost when it was attempted to retain it permanently *in vitro*. All cells soon came to look alike despite the complexity of the original tissue. In view of these difficulties, many morphologists developed a technique of their own. But in most cases such methods have allowed merely a survival of the cells in various media until death finally occurred.

The method to be reported here is an attempt to create a simple technique for the cultivation of pure strains of tissue cells which will more nearly imitate the conditions found in the adult organism. It is an attempt to discover new properties in the cells, properties which may never be detected under conditions of excessive multiplication.

Into a Carrel flask, of the 3½ cm. D-type, is introduced 0.5 cc. plasma and 1.0 cc. Tyrode solution. Coagulation of the plasma is initiated by the addition of one or 2 drops of embryonic tissue juice. The fragment of tissue is placed in the medium as soon as the tissue juice has been added and before coagulation has occurred. The flask is then closed and placed in the incubator. After 2 days the flask is reopened and 2 cc. of Tyrode solution is poured over the surface of the clot. The flask is then returned to the incubator for ½ to 1 hour. At the end of this time the Tyrode solution is aspirated and replaced by 0.5 cc. plasma containing 1% of a 10%

[1] Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 583.

solution of heparin. The flask is again returned to the incubator for 2 or 3 hours. The concentration of the heparin should be just sufficient to keep the plasma in a fluid state over this period. The plasma is next removed and the flask left with a dry clot for another 2 days, when the process is repeated as before. This procedure allows a washing out of accumulated waste products with the Tyrode solution, and a replacement of food materials with the fresh plasma. And if this procedure be carried out regularly, the cells are provided with food materials not very different in quality and quantity from those supplied in the organism.

We have found the rate of proliferation, under these conditions, to be very low. However, cultures which have been so treated, and allowed to remain in the same clot for a month, have, upon histological examination, proved to be in just as healthy a condition as those which were allowed to grow vigorously in embryonic tissue juice according to the usual method. Under these conditions, we have a more ideal relationship between the individual cells of the colony. The cells become dependent upon each other. They adapt themselves to the poorer conditions in such a manner that their metabolism is reduced to a minimum and the rate of proliferation becomes so adjusted that a condition of equilibrium is reached between the supply of nourishment and the elimination of metabolic substances. And finally, it is believed that a condition is ultimately reached where cell death may induce new cell divisions, thus reestablishing the equilibrium.

4409

The Oestrous Hormone in the Urine of Pregnant Cows.*

FREDERICK L. HISAW AND R. K. MEYER.

From the Zoology Department, University of Wisconsin.

Through the kindness of Dr. L. J. Cole of the Genetics Department a number of pregnant cows were placed at our disposal to make a quantitative study of hormones in the urine. One of the points of interest found in this study was the great amount of the oestrous hormone present. A quantitative determination of the oestrous hormone in the urine of pregnant women has been recently

* Assisted by grants from the National Research Council, Committee on Problems of Sex.

reported by Veler and Doisy.[1] Ascheim and Zondek[2] have mentioned the presence of this hormone in the urine of pregnant cows. The data presented in this report are concerned with the quantitative relationship between the oestrous hormone in the urine of cows and the time of pregnancy. Twenty-four hour samples of the urine were collected and the hormone extracted with ether and then taken up in Mazola oil. A second method was also used in which the urine was neutralized, Berkefelded and injected without further treatment. The method of assaying the hormone was that of Allen and Doisy, rats being used as test animals.* The results obtained by these procedures are presented in the accompanying tables. In general the amount of the oestrous producing hormone increases as pregnancy progresses. The data seem to indicate an enormous increase of the hormone in the later days of pregnancy

TABLE I.
Analysis of Urine of Pregnant Cows.

No. of cow	No. days pregnant	No. cc. per 24 hour sample	No. rat units per 24 hours	Remarks
46B	31	14,150	0	Mazola oil
46B	57	9,100	0	" "
60A	44	6,000	41	" "
61B	188	6,800	186	" "
61B	214	2,750	215	" "
71A	128	11,925	307	" "
71A	154	4,000	266	" "
71A	270	7,100	4733	Injection of urine
62B	254	10,325	667	Mazola oil
62B	280	4,050	3242	" "
43D	261	4,450	483	" "
45A	167	6,000	960	Injection of urine
55A	260	5,850	3250	" " "
25C	280	9,950	6636	" " "

TABLE II.
Decrease of the Oestrous Hormone after Parturition.

No. of cow	No. days pregnant	No. cc. per 24 hour sample	No. rat units per 24 hours	Remarks.
62B	280	4,050	3242	Mazola oil prep.
62B	.....	2,800	112	19 hrs. post partum. First sample post partum. Mazola oil prep.
62B	.....	1,325	0	25 hrs. post partum. Second sample post partum. Mazola oil prep.

[1] Veler, C. D., and Doisy, E. A. PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 806.
[2] Zondek, B., and Ascheim, S., *Klin. Woch.*, vii, 1401.
* Three injections of material were made in 36 hours.

with a sudden decrease to little or none within the first 24 hours after parturition. Close analysis of the data shows certain discrepancies between them and the general statement just made. Cows in the same stage of pregnancy do not seem to give the same amount of hormone per day. Further, a cow may give more hormone in the early stages of pregnancy than at a later time as indicated in the case of cow 71A, table I. More data on these and other points are being collected and will be presented at a future time.

4410

The Histologic Effects Produced in Albino Rats by High Frequency Currents.

W. M. BALDWIN AND W. C. NELSON.

From the Albany Medical College, Albany, N. Y.

Beginning in March, 1928, with the advice and help of H. R. Hosmer regarding the physical apparatus employed, a series of experiments was begun, the object of which was to ascertain the histologic effects produced in the animal body by high-frequency currents. A current oscillating at a rate of from 5 to 6 million cycles per second under a current strength of 2 to 4 amperes and a pressure of 3000 volts between the plate electrodes was employed. Anesthetized albino rats interposed between these electrodes, spaced from 3.5 to 6.0 cm., for time intervals varying from 4 to 30 minutes demonstrated a body-temperature rise directly dependent upon the duration of the exposure. Both rectal and abdominal temperatures of as high as 45° were obtained before the death of the rats. The most definite and constant cytologic results were produced in these rats, however, by rayings repeated on alternate days at rectal temperatures of from 40° to 42°, and especially when such body-temperatures were maintained over periods of time varying from 10 to 30 minutes.

Upon autopsy there was observable a definite diminution in the quantity of the body fluids and in blood volume. The ventricles of the heart were filled with clotted blood, usually in complete diastole. The periphery of the lungs, of the heart, and of the liver afforded evidences of an incipient coagulation necrosis. The blood vessels, likewise, in these superficial areas were dilated and filled with clotted blood. Such cytologic changes as were presented by the blood and bone-marrow are to be reported at a later date. The spleen was not

markedly increased in size but on section was found to be choked with red blood cells in various stages of degeneration.

The sinusoids of the liver were dilated and likewise filled with blood cells while leucocytes with fragmented and pyknotic nuclei were conspicuous both within the blood vessels and among the liver cells. Small foci formed by a coagulation necrosis of the liver cells measuring from 1/6 to ¼ the diameter of an hepatic lobule were restricted to and characteristic of the periphery of these lobules. Such foci were invariably associated with the region of the afferent blood vessels.

Extravasations of blood were not infrequent in both cortex and medulla. The glomeruli of the kidney were dilated and the capillaries of the kidney filled with the clotted blood. The presence of altered red blood cells, fibrin and cell detritus seemed not to be inconstant features of Bowman's capsule and of the proximal and distal convoluted tubules. Evidences of a degeneration of the cells lining these convoluted tubules and the ascending loop of Henle were generally present.

The stomach and proximal portion of the duodenum were normal, but beginning with the ampulla of Vater the mucosa of the small intestine was constantly found in a condition of degeneration and exfoliation, the lumen of the gut being choked with this cellular mucosal detritus. This exfoliation approached the level of the submucosa and the fundus of the intestinal glands. The entire thickness of the intact basal portion of the mucosa and the submucosa was infiltrated with leucocytes in various stages of degeneration. Likewise, such cells were found in great numbers in the detritus of the lumen. Small extravasations of blood were noted, chiefly in the submucosa and subserosa. The capillaries of the entire intestinal wall were distended and engorged with blood. There were no evidences of degeneration either in the sex organs or in the endocrine and nervous systems. Where the voluntary muscles had been greatly heated, the change noted was of the nature of a coagulation necrosis. The pancreas appeared normal.

These histologic changes produced by such energy conditions seem to bear out the contentions of Christie and Loomis[1] as opposed to those of Schereschewsky.[2] No evidence was obtained which might afford a basis for the assumption of a selective histologic action of this form of energy. The sharp restriction of the focal necrot-

[1] Christie, R. V., and Loomis, A. L., *J. Exp. Med.*, 1929, xlix, 303.

[2] Schereschewsky, J. W., *Pub. Health Rep.*, 1926, xli, 1939; *Ibid.*, 1928, xliii, 927.

ic areas to the periphery of the liver lobules argues in favor of the presence in the blood stream of some toxinous material (as yet unidentified), which produces a necrosis in those cells of the liver lobule first encountered by this afferent blood. The necrotic mucosal conditions observed in the intestine, beginning at the ampulla of Vater and extending to the proximal portion of the large intestine, point towards an altered chemical constitution of the bile rather than pancreatic secretion. Deductively this seems referable rather to the altered hepatic features in view of the absence of any observable histologic pancreatic changes. The kidney changes may well be referred to the elimination function of this organ as regards the altered chemical constituent of the blood. There exists a striking parallelism between the conditions observed in these experiments and those noted as the result of extreme burns from various sources of external heat and, therefore, seem to substantiate the conclusions of Christie and Loomis that the changes effected are the result of a true heating condition of the body brought about directly as the result of the oscillating current.

4411

The Transportation of Carbon Dioxide by Paraffine Oil and Some Other Substances.

SAMUEL E. HILL. (Introduced by E. Newton Harvey.)

From the Physiological Laboratory, Princeton University.

In a previous paper[1] the use of luminous bacteria as an oxygen indicator was described. Some rough estimates of the passage of oxygen through a number of substances were given, as determined by the length of luminescent columns of bacteria. The properties of several of these substances, notably dry collodion, were of interest and these and some others were selected for further study with reference to carbon dioxide. In this case the time in hours required for one atmosphere of carbon dioxide to decolorize 1 cm. of phenolphthalein in NaOH agar is used for comparison. It is clearly recognized that this is not a quantitative method for the study of diffusion as there is a concentration gradient of carbon dioxide in the colorless portion of the phenolphthalein as well as through the membrane closing the tube. Nevertheless, it makes a very striking demonstration for class use and does give a qualitative expression for

[1] Hill, Samuel E., *Science*, 1928, lxvii, 374.

the passage of carbon dioxide. The proper methods of quantitative study may be found in the papers of Krogh[2] and of Northrop,[3] to mention only two.

In the earlier experiment temperature control was relied on to minimize convection currents, since the experiments could run for only a short time. With the phenolphthalein, NaOH, agar system the agar was used to prevent convection currents in the solution into which the carbon dioxide diffused, but it at once became evident that convection currents in the lighter oils were assisting in the transportation of carbon dioxide, and a method was sought for reducing or eliminating them. The most efficient found was that of cutting disks of blotting paper which exactly fitted the inside of the test tubes used, saturating these with paraffine oil, and packing them tightly in the tube to the required depth. An easier method and one almost as efficient was that of saturating cotton with oil and packing it in the tube. The difference in the relative amounts of carbon dioxide carried by the oil when convection currents were permitted and when they were restrained may be seen by reference to the table.

TABLE I.

Time required in hours for carbon dioxide at one atmosphere pressure to change color of indicator in 1 cm. of test solution.

EXP. 1.		EXP. 2.	
Open control	13	Open control	12
Paraffine oil, 2 cm.	15	Dry collodion	13
Paraffine oil, 1.5 cm.	16	Rubber dam	13.5
Light oil, 1.5 cm.	22	Thicker rubber dam	18
Heavy oil, 1.5 cm.	60	Paraffined rubber dam	49
Rubber stopper, 1.5 cm.	81	Beeswax	102
Paraffine oil in blotting paper, 1.5 cm.	115	Sealing wax	
Water in blotting paper, 1.5 cm.	36	Solid paraffine, 1 cm.	
Paraffine oil in cotton, 1.5 cm.	112	Vaseline, 1 cm.	
Water in cotton, 1.5 cm.	35	DeKhotinsky cement	
Cotton, 1.5 cm.	14	Picein cement	
		Paraffined cork	
		2 mm. Paraffine on linen	

In general those substances found impermeable or relatively impermeable to oxygen were impermeable to carbon dioxide also, the most striking differences being observed with rubber and dry collodion. A trace of passage of oxygen through dry collodion was found and a trifle more through rubber, whereas carbon dioxide passed with the greatest ease through both.

[2] Krogh, August, *J. Physiol.*, 1919, lii, 391.

[3] Northrop, John H., *J. Gen. Physiol.*, 1927, xi, 233; 1929, xii, 435.

Kruse,[4] in a study of oxygen exclusion by various oils, ascribed the greater efficiency of the heavier oil to its higher viscosity. In the present study, the introduction of cotton or blotting paper reduces convection currents to a minimum without the high viscosity associated with heavy oils. The rate of passage of carbon dioxide through the heavy oil is then greater than through paraffine oil, whereas when convection is permitted the paraffine oil carries the greater quantity. When high viscosity and absence of convection currents are associated, as in the case of vaseline and solid paraffine, the rate of transportation of carbon dioxide becomes negligible.

The substances used to close the tubes were less than 1 mm. thick except where otherwise noted. The tubes were kept in an atmosphere of carbon dioxide for 14 days, and those substances which allowed no carbon dioxide to pass in that time may be considered impervious under any ordinary conditions. Rubber, as noted by many previous observers, is quite worthless against carbon dioxide, as even a rubber stopper 1.5 cm. thick allowed appreciable amounts to pass.

4412

Antitryptic Titre in Pregnancy and in Hyperthyroidism.

LOUIS B. FLEXNER, JOSEPH BERKSON, HYMAN WINTERS AND IRVING WOLMAN. (Introduced by Raymond Pearl.)

From the Department of Obstetrics and the Institute for Biological Research of the Johns Hopkins University.

Since it is known that the normal blood serum contains substances which inhibit the action of enzymes, and that the titre of such substances varies, there is ground for supposing that this property of the blood plays some physiological rôle. Specifically it might be conjectured that it affects catabolism and that in conditions where changes in metabolism are an outstanding feature correlated changes in antienzyme titre would be found, and that these might have etiological import. Indeed, fragmentary observations of changes occurring in pathological conditions have been made.[1, 2, 3]

With this thought in mind we investigated two conditions in which metabolic changes were prominent: (1) pregnancy, (2) toxic hyper-

[4] Kruse, T. K., *J. Phar. and Exp. Ther.*, 1926, xxvii, 243.

[1] Meyer, K., *Berl. klin. Wchnschr.*, 1909, xlvi, 1064.

[2] Gräfenberg, E., *München. med. Wchnschr.*, 1909, lvi, 702.

[3] Thaler, H., *Wien. klin. Wchnschr.*, 1909, xxii, 850.

thyroidism. The method used follows Northrop and Hussey.[4] 5 cc. of a 1% solution of commercial pancreatin were mixed with 0.5 cc. of serum to be tested; 0.5 cc. of the mixture was then added to 10 cc. of a 3% solution of commercial gelatin in an Ostwald viscosimeter and the viscosity changes noted at 35° C. Two of us have shown elsewhere[5] that under these conditions the viscosity changes may be represented as a logarithmic function and that the exponential rate is linearly related to enzymatic activity. Accordingly the exponential rate of viscosity change was obtained for each serum examined and compared with one or more normal sera observed at the same time. As a measure of the antitryptic titre we use the difference between the rate obtained for the experimental serum, and that obtained for the normal serum which was run in the same bath, and this expressed as a percentage of the control rate, a plus sign indicating an experimental serum the titre of which is greater and a negative one which is less than the normal control.

A preliminary examination of 10 normal sera taken at different times of the day indicated a comparative stability of the measure of antitryptic titre. A mean value of 20.2 was obtained in this series and a standard deviation of 2.1 yielding therefore a coefficient of variation of 10.4%. The determinations comprising this series are independent of the control normals which were set severally against the runs made with the experimental sera.

The results for the pregnancy series follow: 159 separate determinations were made. All but 10 of these showed an antitryptic titre greater than normal. Expressing the time in the pregnant period when the serum was obtained as weeks prior to delivery, the mean time for the 149 cases showing a plus titre was 8.9, the standard deviation 8.42. For this same group the mean antitryptic titre was plus 34.95% ±0.92, the standard deviation 16.61% ±0.65 and the coefficient of variation 47.5%. From this we may say that about two-thirds of such cases fall within plus 18% and plus 52% in the measure of titre used. Graphically (Fig. 1) we show the mean titre at various times in the period of pregnancy for 119 of the 159 cases in which the time of delivery was obtainable. It would appear from this that there is a progressive rise of titre from conception until about 6 lunar months before delivery, after which there is a decrease. The small number of cases involved, particularly for the early months, makes the evidence that a cyclic change in titre occurs during pregnancy only suggestive, but that there is a rise

[4] Northrop, J. H., and Hussey, R. G., *J. Gen. Physiol.*, 1922-23, v, 353.

[5] Berkson, J., and Flexner, L. B., *J. Gen. Physiol.*, 1928, xi, 441.

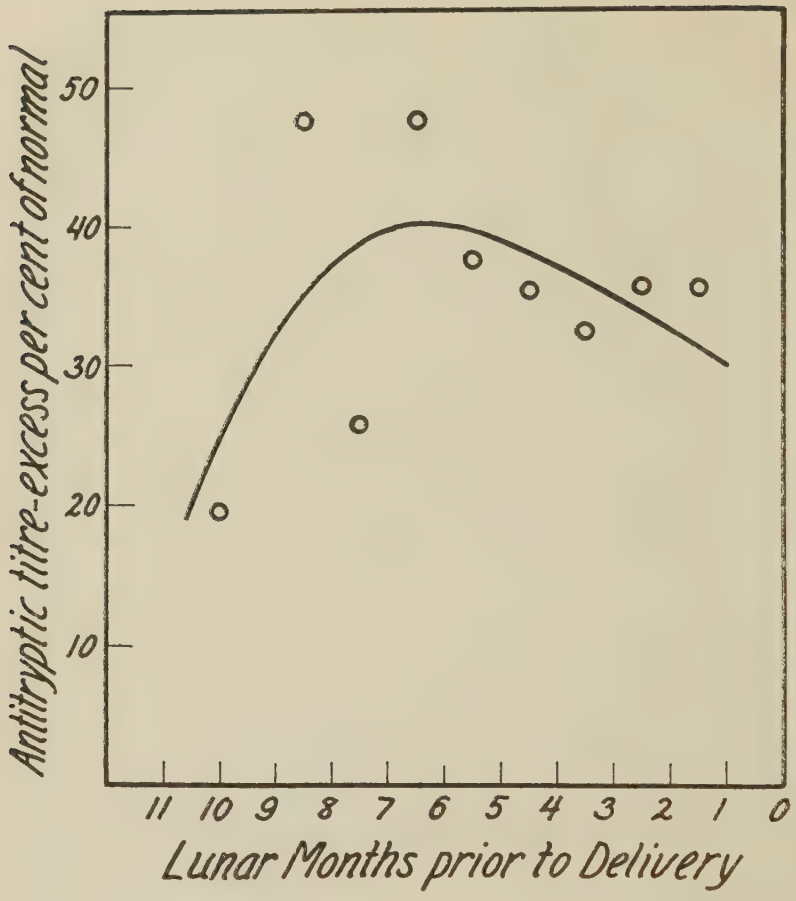

FIG. 1.

from conception to a point well above normal seems conclusively established. Whether this fact can be utilized for the diagnosis of pregnancy is yet to be determined.

The Antitryptic Titre in Hyperthyroidism. Twenty-five sera of patients diagnosed definitely as suffering from hyperthyroidism and all showing increased basal metabolic rates were examined in the same way as those in the pregnant series described above. All showed an antitryptic titre above the normal. The mean value was $+32.0\% \pm 2.8\%$, the standard deviation $20.5\% \pm 2.0\%$, the coefficient of variation 64.0%. This may be interpreted as indicating that about two-thirds of such cases as those examined have a titre between plus 12% and plus 52%.

Twelve of these cases are identical with ones in the series reported

by Nicholls and Perlzweig[6] and for which they reported decreased total and unchanged unsaturated fats. This militates against the conclusion of Jobling and Peterson[7] that the antitryptic substances of the blood serum are identified in the unsaturated fatty acids.

4413

Impairment of the Birth Mechanism Due to Hormones from the Anterior Hypophysis.*

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From the Department of Anatomy, University of California.

It has been shown in this laboratory[1] that the administration of the hypophyseal growth hormone (alkaline extracts of bovine hypophysis) during the last week of pregnancy in some way injures the birth mechanism. Treatment is stopped on day 21. There is then a characteristic prolongation of pregnancy of from 2 to 3 days, with final birth of dead or living foetuses. There are also instances of complete failure even to give birth to dead foetuses and of the consequent resorption of the latter. The effect has been shown to be due in all probability to changes which the treatment provokes in the ovaries of the pregnant animal, more especially to the formation of fresh lutein tissue there.[2]

Now another hormone of the anterior hypophysis, one of the outstanding characteristics of which is the production of precocious maturity in young female rodents, strangely enough, in addition to its marked stimulation of the follicular apparatus also causes the luteinization of follicle walls, a point strongly emphasized by Zondek and Aschheim.[3] It might be expected, therefore, that whatever effects from growth hormone treatment are due to the creation of fresh lutein tissue in the ovary would also be encountered in the treatment of animals with this second hormone from the anterior hypophysis.

We propose to show that this is the case as regards interference

[6] Nicholls, E. G., and Perlzweig, W. A., *J. Clin. Inv.*, 1928, v, 181.

[7] Jobling, J. W., and Peterson, W., *J. Exp. Med.*, 1914, xix, 459.

* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

[1] Teel, H. M., *Am. J. Physiol.*, 1926, lxxix, 170.

[2] Dispensa, J. G., M.A. Thesis, University of California, May, 1928 (unpublished).

[3] Zondek, B., and Aschheim, S., *Deutsche med. Wchschr.*, Feb. 19, 1926; *Ztschr. f. Geburts. u. Gynäk.*, 1926, xc, 372, 387.

with the mechanism of birth. Engle[4] has recently doubted the existence of these effects. His method consisted in daily implantation into the pregnant animal of anterior hypophyseal tissue—the most effective method, it must be admitted, of provoking the precocious maturity of young female rats and mice. The characteristic prolongation of gestation can be secured by anterior hypophyseal implants, for instance, by 1 or 2 glands daily for the last 5 days of gestation. By administering a single hypophyseal implant from a gonadectomized male every other day beginning on day 12 of gestation, the birth mechanism was destroyed in 8 of 10 cases. Extracts of the ripe human placenta and of the urine of pregnant women† gave us the opportunity for another type of dosage with this hormone. For many months we have had such extracts in hand, solutions proven to contain neither folliculin nor the growth hormone.‡ With these we have secured the same interference with the birth mechanism in pregnant rats so characteristic of treatment with the growth hormone.

We would emphasize the fact that, unlike the invariable effect of the growth hormone, the administration of the maturity hormone does not hurt the birth mechanism in all cases. In the case in which the maturity hormone was administered by the implant method and hence greatly stimulated the follicular apparatus, folliculin was, of

[4] Engle, E. T., and Mermod, C., *Anat. Record*, 1928, xxxviii, 11.

† Our extracts from the urine of pregnant women are prepared by methods which we find resemble closely those just published by Biedl (*Endokrinologie*, 1928, ii). The urine is slightly acidified (green-blue to Brom Thymol Blue), filtered and concentrated to 1/8 volume in vacuo (35° C.) and filtered; ethyl alcohol is added to 75%. The mixture is centrifuged and the residue dissolved in water. The precipitation from 75% alcohol is repeated twice. The solid matter left after the third alcohol precipitation is dissolved in water, centrifuged, and the supernatant fluid dialyzed 16 hours. (Sometimes the extract has been evaporated to dryness and extracted twice with ether, then redissolved in water. This step was inserted to make doubly sure of the absence of folliculin.) The extract obtained is colorless and clear, gives no biuret reaction, has total solids of 0.2 gm per 100 cc. and when injected intramuscularly or subcutaneously is able to mature 24 day old rats in 1/16 cc. daily dose for 3 days. The placenta extracts though less pure have been equally potent (3/16 cc. total dose). They have been prepared by extracting fresh (or frozen) human placenta with weak acid (500 gm. ground placenta + 800 cc. water + 160 cc. 0.2/N acetic acid). Folliculin was removed by extraction with ether.

‡ A total of 9 cc., for instance, (distributed in 9 doses) has been injected subcutaneously in spayed females over a period of 2 days without provoking oestrous smear changes. The same solution provoked the maturity of 24 day old rats within 4 days when given in 3 daily doses of 1/16 cc. each. Nor was there evidence that the solution contained the growth hormone, as tested by its administration in 2 cc. daily quantities intraperitoneally to 5 months old normal females.

course, produced in considerable quantity in the affected gonad. It is to the coincident presence of this hormone that we would attribute the normality of the birth mechanism which we have encountered in high dosage with the implant method (8 rat glands per day). It does, in fact, occasionally bring about premature birth through a folliculin effect. On the other hand, the administration only once daily of a highly concentrated fraction out of human placenta or urine of pregnant women has given the characteristic birth retarding effect.

4414

Stimulation of Placentoma Reaction in Virginal Endometrium by Treatment with Anterior Hypophyseal Hormone.

HERBERT M. EVANS AND MIRIAM E. SIMPSON.

From the Department of Anatomy, University of California.

It has been shown that the anterior hypophyseal growth hormone markedly aids the placentoma reaction of the endometrium of adult virginal rats, tumors of this sort being provoked about silk threads when simultaneous dosage with the growth hormone is carried out.* The anterior hypophyseal hormone which promotes precocious development of the immature ovary, has a similar effect if it is not administered so as to stimulate greatly the follicular apparatus and lead to the formation of much folliculin. It is for this reason, we believe, that the administration of forms of the hormone (extracts of the urine of pregnant women and of human placenta) are more efficacious in stimulating placentomata than is the implant method.

4415

Hyperplasia of Mammary Apparatus in Precocious Maturity Induced by Anterior Hypophyseal Hormone.

HERBERT M. EVANS AND MIRIAM E. SIMPSON.

From the Department of Anatomy, University of California.

Within 6 days, the mammary tree of 24 day old female rats is clearly increased in complexity by all methods of administration of

* Whatever the substance injected, it is a routine to give 3 days preliminary treatment and 5 days treatment after insertion of the threads, autopsy occurring on the sixth day after the threads were inserted.

the anterior hypophyseal hormone known to us. We have employed bovine and rat implants and folliculin-free and growth hormone-free extracts of the ripe human placenta and of the urine of pregnant women. The implant method shows such an effect with only 2 daily treatments and sacrifice on the fourth day. In some cases hyperplasia was seen to have begun before lutein structures were present in the young ovaries. The secretion of corpora lutea therefore could not have been responsible for this stimulus to mammary growth, though the growth was always most marked in cases in which corpora lutea were coincident with a hypertrophied follicular apparatus.

4416

Hyperplasia of Mammary Apparatus of Adult Virginal Females Induced by Anterior Hypophyseal Hormones.

HERBERT M. EVANS AND MIRIAM E. SIMPSON.

From the University of California, Department of Anatomy.

The mammary tree of adult virginal rats invariably undergoes some hyperplasia after a month of daily treatment with alkaline extracts of the anterior hypophysis, solutions very rich in growth hormone and almost completely devoid of the hormone which stimulates the ovaries of immature rodents to precocious development. By more chronic treatment with such an extract this can be brought to true milk secretion. Mammary hyperplasia takes place with more speed after treatment with the anterior hypophyseal ovary-stimulating hormone (bovine or rat hypophysis implant, or extracts of placenta and of the urine of pregnant women). The hyperplasia is, in fact, remarkable after only 10 days dosage with the latter methods.

4417

Effects of Thyroparathyroidectomy on the Teeth of the Rat.

FREDERIC T. JUNG AND WILLIAM G. SKILLEN. (Introduced by A. C. Ivy.)

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The effects observed by Erdheim[1] and later by Toyofuku[2] in the teeth of parathyroidectomized white rats led Erdheim to emphasize

[1] Erdheim, J., *Frankf. Z. Path.*, 1911, vii, 175.

[2] Toyofuku, T., *Ibid.*, 1911, vii, 249.

the relation of the parathyroids to calcium metabolism. The uncertainty as to the mode of action of the parathyroids caused us to undertake a similar study of the teeth of some thyroparathyroidectomized rats which had been produced for another purpose.

The two rats whose teeth were studied were males and had been kept, like the others, on a standard diet of 60% cracked corn, 20% powdered milk, 16% powdered casein, 3% alfalfa meal, 0.5% sodium chloride, and 0.5% calcium carbonate. Both showed typical tetany after the operation, and both showed gross dental defects before death. Rat 1 had a brown line along the anterolateral edge of the right upper incisor when it died on the 29th day; rat 2 had suffered a spontaneous fracture of both lower incisors and marked elongation of the opposing upper incisors before it died on the 82nd day. Microphotographs, with detailed descriptions, of the transverse sections of the teeth of these rats will be published elsewhere.[3] The following is a brief summary of the findings:

RAT 1. Pulp and odontoblasts well preserved, dentine well calcified. The greatest disturbance occurred in the enamel epithelium and in the bone adjacent to one of the teeth. The ameloblasts were disorganized in places, and there was proliferation of the outer layer of the enamel epithelium. These layers showed some folding. At one level a striking exostosis of the alveolar bone had taken place. This abnormal bone growth obliterated the peridental membrane as it progressed inwards towards the tooth, and it finally lay in contact with the enamel itself. In so doing it either pushed aside or induced atrophy of the enamel epithelium in its path.

RAT 2. Conditions varied greatly from level to level, but were abnormal throughout. Pulp showed some congestion, vacuolation of the intercellular substance, various degrees of atrophy of the odontoblasts, and sporadic calcareous nodules. Capillary canals running from the pulp into dentine were abnormally numerous. Dentine was in the main under-calcified, and showed a zone of fracture; but there were zones of over-calcification also. In general the dentine was normal in contour and thickness. The enamel was most irregular. In places it was very poorly calcified, leaving a conspicuous organic matrix in the sections. In other places it disappeared, so that the space which it ought to have occupied was filled with peridental tissue and with disorganized masses of enamel epithelium in various stages of proliferation, disarray, and atrophy. Marked folding occurred at some levels. The alveolar bone showed exostoses, disorganizations, atrophied bone cells, and irregular calcifi-

[3] Skillen, W. G., and Jung, F. T., *Am. Dent. J.*, 1929.

cations (i. e., both under and over-calcification, as judged from the staining).

These findings do not differ essentially from those reported by Erdheim and Toyofuku (who removed only parathyroids), but do contradict a statement by Gies.[4] An interpretation of the data will be attempted in our final paper.

4418

Ultrafiltration Studies on the Virus of Poliomyelitis.*

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From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

Ultrafiltration studies on so-called filterable viruses, including fowl-plague, vaccinia, herpes, foot-and-mouth disease and mosaic diseases of plants, have been reported by Andriewsky,[1] Doerr and Pick,[2] Levaditi and Nicolau,[3] Duggar and Karrer-Armstrong,[4] Levaditi, Nicolau and Galloway,[5] Olitsky and Boëz,[6] Zinsser and Tang,[7] Berger,[8] but to our knowledge no such investigations have thus far been reported on the virus of poliomyelitis. While the filterability of this virus through Berkefeld candles has been reported (Landsteiner and Levaditi,[9] Flexner and Lewis[10]) it is still not known whether the etiological agent is a small bacterium or a true ultrascopic virus. With this in mind we have undertaken a program of investigation designed to elicit more accurate information as to the magnitude of the organism concerned. The present paper deals with some of our observations thus far.

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[4] Gies, W. J., and collaborators, *J. Natl. Dent. Assn.*, 1918, v, 527.

* Aided by the Mrs. Mary Hooper Somers Medical Research Fund.

[1] Andriewsky, P., *Centralbl. f. Bakteriol.*, Abt. I., Orig., 1915, lxxv, 90.

[2] Doerr, R., and Pick, R., *Ibid.*, 1915, lxxvi, 476.

[3] Levaditi, C., and Nicolau, S., *Compt. rend. Acad. des Sci.*, 1923, clxxvi, 717.

[4] Duggar, B. M., and Karrer-Armstrong, J., *Ann. Missouri Bot. Gard.*, 1923, x, 191.

[5] Levaditi, C., Nicolau, S., and Galloway, I. A., *Compt. rend. Acad. Sci.*, 1926, clxxxii, 247.

[6] Olitsky, P. K., and Boëz, L., *J. Exp. Med.*, 1927, xlv, 685.

[7] Zinsser, H., and Tang, E. F., *Ibid.*, 1927, xlvi, 357.

[8] Berger, E., *Z. Hyg.*, 1928, cviii, 315.

[9] Landsteiner and Levaditi, *Compt. rend. Soc. Biol.*, 1909, lxvii, 592.

[10] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1909, liii, 2095.

Our experiments were carried out with a highly virulent strain of poliomyelitis virus (Aycock strain). The virus suspensions were prepared by grinding the recently harvested cord and medulla of poliomyelitis monkeys in a mortar,† in the presence of quartz sand, for at least one hour.. The material was then made up into a 5% suspension in neutral physiological saline. Before filtration the suspensions were centrifuged at moderate speed for 5 to 15 minutes to remove the larger particles. The reaction of the suspension after centrifugation was then determined by a colorimetric method and was found to be between pH 5.80 and pH 5.90.

The membranes through which the suspensions were filtered were prepared by dipping Whatman No. 1 filter paper into glacial acetic acid containing various percentages of dried Anthony's negative cotton. After washing them in successive changes of sterile distilled water, to free them of the acetic acid, they were mounted between the ground glass surfaces of 2 opposing cylinders of a special filter device.‡ These membranes were carefully checked for defects both before and after each experiment. They were graded on the basis of their permeability to colloidal particles of known charge and particle size and on their permeability to water (data based upon Poisseuille's formula). The filtrations were all carried out under low negative pressures (0.5, 1.0 or 1.5 cm. Hg). The H-ion concentration of the filtrates ranged between pH 5.80 and 5.90. The monkeys received the respective filtrates in single doses of 2 cc. injected into the frontal lobe of the brain.

No difficulty has been experienced in producing the typical disease in monkeys inoculated with filtrates obtained by passing the suspensions through 2 superimposed 0.5% colloidin membranes, though these filtrates appeared water-clear, or at most slightly opalescent. Four monkeys inoculated with 4 different filtrates have succumbed to the disease within the usual period of time. It has not been possible, however, to get "takes" with filtrates obtained by directly passing the crude virus suspensions, even after centrifugation, through denser membranes (1.0%, 1.5%, 2.0% and 2.5%).

These results might suggest a relatively large magnitude for the virus or virus-bearing particle were it not for the fact that it is possible to put it through denser membranes after it is freed of most of the colloidal matter by a preliminary filtration through 2 superimposed 0.5% colloidin membranes. By doing this we have been able to get repeated "takes" with filtrates through 1.0%, 1.5% and

† Driven mechanically in a special machine designed by Schultz and Banham.

‡ A description of this filtering apparatus will be published in another paper.

2.0% membranes. Thus far less permeable membranes have completely retained the virus. While our experiments do not place the virus definitely in the ultravisible group, there remains a possibility that with further perfection of the experimental procedure the virus may prove capable of traversing membranes of even greater density.

The 2% membranes in question retained completely cultures of *B. prodigiosus* as well as aqueous and broth suspensions of various Streptococci. The membrane series used in these experiments has been carefully studied by one of us (A. P. K.) along the lines suggested by Hitchcock,[11] Walpole,[12] and others. The results of this particular investigation of the membranes themselves will be reported later. While we are not in a position to definitely fix the lower limits of filterability of poliomyelitis virus, because of the disturbing influence of associated colloids, the results obtained thus far indicate that the magnitude of the virus lies below 300$\mu\mu$. From our observations it appears that vaccinia (Levaditi) and herpes (Goodpasture) viruses, employed in the form of virus brain suspensions and filtered under comparable conditions, are no more filterable than the virus of poliomyelitis. There can be no question that such factors as the association of virus particles with protein aggregates and the adsorption of such aggregates, or of free virus corpuscles, at the pore surfaces deserve further study. The influence of proteins and protein derivatives on ultrafiltration is clearly indicated by the results recently reported by Krueger and Tamada,[13] in connection with similar studies on the bacteriophage. Ultrafiltration experiments carried out with virus brain or cord suspensions are even further complicated by the high lipoid content of the menstrum.

It is significant that aerobic and anaerobic cultures made from the filtrates injected failed to reveal the presence of streptococci or other microorganisms.

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[11] Hitchcock, D. I., *J. Gen. Physiol.*, 1925, ix, 755.

[12] Walpole, G. S., *Biochem. J.*, 1915, ix, 284.

[13] Krueger, A. P., and Tamada, H., PROC. SOC. EXP. BIOL. AND MED., 1929, in press.

4419

Influence of the Maternal Diet on Concentration of Hemoglobin of Nursing Young of Albino Rat.*

BARNETT SURE AND M. C. KIK.

From the Laboratory of Agricultural Chemistry, University of Arkansas, Fayetteville.

In a recent communication[1] we have reported on "Blood Formation of the Albino Rat on a Standard Dietary Regime", of the following composition: whole wheat, 25; rolled oats, 26; yellow corn, 25; oil meal, 15; commercial casein, 5; cod liver oil, 1; $CaCO_3$, 0.5; NaCl, 0.5; and a liberal supply of whole milk daily. On that ration, designated as stock diet 1, we found the concentration of hemoglobin of nursing baby rats appreciably below that reported by Williamson and Ets.[2] We are now finding considerably higher values on a different type of diet, designated as ration 1145, of the following composition: casein (purified) 20; dehydrated baker's yeast (Northwestern), 10; McCollum's salt mixture No. 185, 4; butter fat, 5; and dextrin, 61. Our findings are submitted graphically in Chart I.

All the lactating mothers were raised on stock diet 1. During the reproduction period the females were divided into 3 groups. One group was mated and allowed to rear the young on stock diet 1; another group was transferred during the later part of pregnancy to ration 1145; and the third group was transferred to ration 1145 on the date of the birth of young.

The curve of hemoglobin concentration of nursing young on maternal stock diet 1 represents 416 determinations.[1] For our work on maternal diet 1145 we made 432 determinations, 18 young having been taken for each age. Since we found no significant increase in the concentration of hemoglobin of the nursing young, whose mothers received ration 1145 during the latter part of gestation compared with that of the nurslings whose mothers received the same diet on the date of birth of the litters, we are showing only the figures of the latter group.

In this study we have also investigated the effect of the above-mentioned 2 types of maternal diets on the concentration of erythrocytes of nursing young of the albino rat. We found that, while the

* Research Paper No. 94, Journal Series, University of Arkansas.

[1] Sure, B., Kik, M. C., and Walker, D. J., *J. Nutr.*, 1929, i, 299.

[2] Williamson, C. S., and Ets, H. N., *Am. J. Physiol.*, 1926, lxxvii, 480.

concentration of red blood corpuscles is somewhat higher on ration 1145 than on stock diet 1, the differences are relatively small compared with the differences in the concentration of hemoglobin of nurslings on these 2 rations.

On ration 1145 we have found marked variations in growth of nursing young. Recently[3] a number of litters showed greater

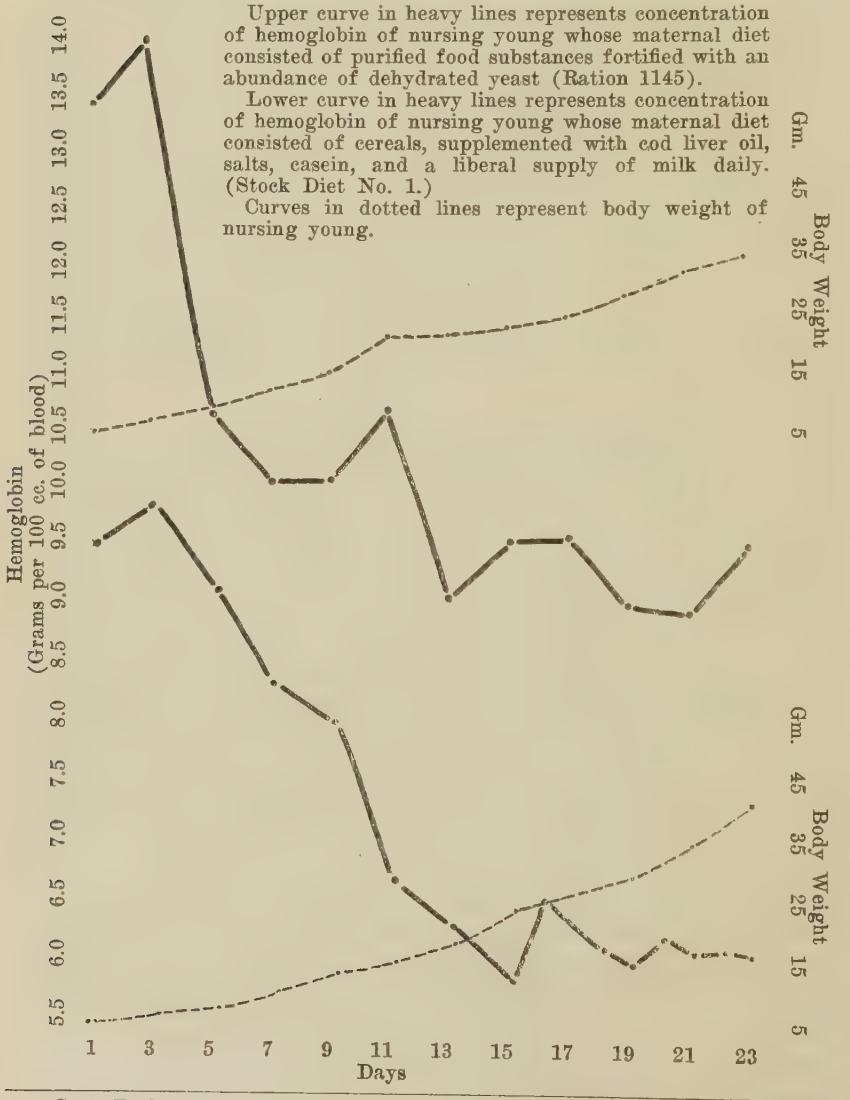
CHART I.

Effect of the Maternal Diet on the Concentration of Hemoglobin of Nursing Young of the Albino Rat.

Upper curve in heavy lines represents concentration of hemoglobin of nursing young whose maternal diet consisted of purified food substances fortified with an abundance of dehydrated yeast (Ration 1145).

Lower curve in heavy lines represents concentration of hemoglobin of nursing young whose maternal diet consisted of cereals, supplemented with cod liver oil, salts, casein, and a liberal supply of milk daily. (Stock Diet No. 1.)

Curves in dotted lines represent body weight of nursing young.



[3] Sure, B., Kik, M. C., and Walker, D. J., *J. Biol. Chem.*, 1929, in press.

growth on such a maternal diet than the nursing young reared on stock diet 1. The nursing young represented in this study on maternal ration 1145, while growing at a more rapid rate during the first half of the lactation period, showed inferior growth during the later part of lactation than the nurslings on maternal stock diet 1. The young of the former group, nevertheless, show higher concentration of hemoglobin throughout the nursing period than the young of the latter group.

4420

The Specific Conductivity of Protozoan Cultures.

E. H. SLIFER, E. C. HERBER, R. BLUMENTHAL, T. P. SUN AND C. C. WANG. (Introduced by J. H. Bodine.)

From the Zoological Laboratory, University of Pennsylvania.

It is an established fact that rhythmical fluctuations of protozoan fauna occur in ponds and laboratory cultures. A number of factors, such as food and oxygen supply, hydrogen ion concentration, temperature, etc., have been measured and attempts made to explain the abundance or scarcity of protozoan forms as being controlled by some one of these factors. Darby[1] has recently presented a thorough review of the subject.

In the present study, one of the factors which seems to have been neglected has been studied. The specific conductivity of several types of laboratory cultures has been measured daily over a considerable period of time. The cultures were made up in duplicate sets with boiled pond water. Cultures I-A and I-B contained dry hay; cultures II-A and II-B had boiled hay and cultures III-A and III-B had no hay at all. All cultures were inoculated from the same stock and kept in the laboratory.

The accompanying graph shows the results obtained. During the first 10 days after the cultures were made, the specific conductivity fluctuated to a considerable degree. From this time on, the points seem to suggest a rhythmical fluctuation such as suggested by the solid lines. Cultures III-A and III-B which had no hay in them and in which the protozoa soon disappeared, showed a specific conductivity which was very low and very constant. Cultures I-A and

[1] Darby, H. H., 1929, *Arch. f. Protist.*, B. 65, S. 1.

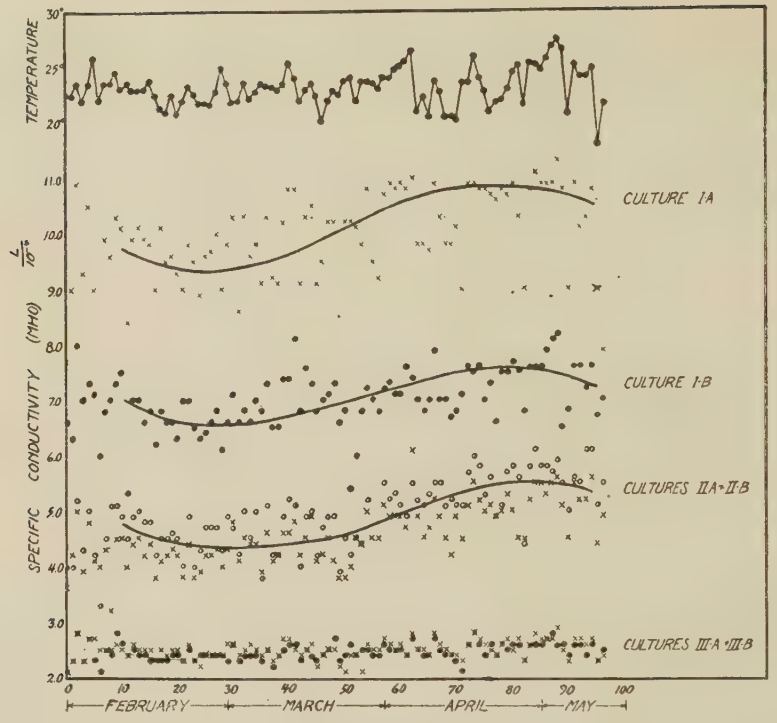


FIG. 1.

Explanation—Cultures I-A and I-B dry hay. Cultures II-A and II-B boiled hay. Cultures III-A and III-B no hay. The solid lines have been drawn as the apparent best fit through the points.

I-B, containing dry hay, had the highest conductivity and showed the greatest fluctuation.

Each set of cultures, although varying one from the other in their degree of conductivity, maintained their relative positions throughout the length of the experiment. The readings of duplicate cultures paralleled each other. In general, the fluctuations seemed to be independent of the temperature unless there was a very sudden and marked change in the temperature.

4421

Increase in Guanidine-like Substance in Acute Liver Injury and Eclampsia.*

A. S. MINOT AND J. T. CUTLER. (Introduced by Paul D. Lamson.)

From the Department of Pharmacology, Vanderbilt University Medical School.

A recent publication from this laboratory[1] reported an increase in guanidine or guanidine-like substances in the blood of dogs during the intoxication produced by carbon tetrachloride and chloroform. The increase in guanidine was soon followed by a marked fall in blood sugar which often reached levels of extreme hypoglycemia. Aside from these changes and a retention of bile pigments no marked abnormalities were noted in the blood chemistry. These intoxications were further characterized by nervous hyperexcitability followed by depression, and a gastro-intestinal irritation evidenced by vomiting, diarrhea, and frequent hemorrhages. The highly protective action of calcium salts in preventing and treating these intoxications has also been emphasized.[1, 2, 3]

The outstanding pathological change produced by chloroform and by carbon tetrachloride, which was the drug chiefly studied, is a severe central necrosis of the liver. Although no definite relationship between the increase in guanidine and the liver damage has yet been demonstrated, it is natural to associate, at least tentatively, the disturbed metabolism with so conspicuous an injury. With this hypothesis in mind, it seems probable that when similar liver injury has been caused in some way other than by the administration of these drugs, similar abnormalities and symptoms may be looked for, and if present might also be relieved by calcium therapy.

Studies of an introductory nature have been made of the blood chemistry with special reference to guanidine and blood sugar levels in a few clinical cases representing various types of liver disease and also in cases of eclampsia—a condition which the majority of observers believe is accompanied by pathological changes in the liver.[4]

* This investigation is one of a series of studies being made under the direction of Dr. P. D. Lamson on the pharmacology and toxicology of carbon tetrachloride. The work is being carried on with the support of the International Health Board.

[1] Minot, A. S., and Cutler, J. T., *J. Clin. Invest.*, 1928, vi, 369.

[2] Minot, A. S., and Cutler, J. T., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 138.

[3] Minot, A. S., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 617,

[4] Stander, H. J., *Medicine*, 1929, viii, 1.

Studies in Cases of Liver Disease. The guanidine was determined by the method of Major and Weber,[5, 6] taking into consideration the possibility of interference due to creatine. The non-protein nitrogen constituents, except uric acid, were determined by the methods of Folin and Wu,[7] uric acid and blood sugar by Benedict's methods.[8, 9]

In 5 cases of chronic liver disease including carcinoma of liver, syphilitic hepatitis, an old alcoholic cirrhosis, and obstructive jaundice, no significant deviation from the normal level was found in any of the blood constituents studied. The cases are of course too few to justify any conclusion, but one would perhaps expect more readjustment to, and hence less metabolic disturbance from an injury developing over a period of months or years than to an equally severe damage produced in a short time. Some indication that this is true is furnished by positive findings in 2 more acute cases. One case was diagnosed as acute arsphenamine hepatitis or acute catarrhal jaundice. On admission this patient showed a fasting blood sugar of 50 mgm., with a moderately increased guanidine concentration. He was mentally depressed and confused. On a regime of oral calcium lactate administration and a diet without meat and high in carbohydrate the mental condition improved and the patient felt much better. The blood sugar level was promptly restored and maintained at a level between 90 and 100 mgm. As the liver condition improved the guanidine concentration gradually returned to normal and calcium administration was no longer necessary to maintain the blood sugar level.

Similar results were obtained temporarily in an infant with congenital abnormality of the liver. No bile entered the intestine except at rare intervals despite the fact that the ducts were patent. Pathological studies after death showed a badly necrosed liver. On admission the baby was markedly jaundiced and showed typical symptoms of tetany. The blood sugar was 57 mgm. and the guanidine concentration about twice the normal level. Oral glucose administration together with the usual diet failed to raise the blood sugar above 70 mgm., but when calcium lactate was also given the blood sugar was maintained between 80 and 90 mgm. for a period of 2 or 3 weeks and the tetanic symptoms disappeared entirely.

[5] Major, R. H., and Weber, C. J., *Johns Hop. Hosp. Bull.*, 1927, xl, 87.

[6] Major, R. H., and Weber, C. J., *Arch. Int. Med.*, 1927, xl, 891.

[7] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, xxxviii, 81.

[8] Benedict, S. R., *J. Biol. Chem.*, 1922, lxi, 187.

[9] Benedict, S. R., *J. Biol. Chem.*, 1926, lxviii, 759.

During this time the calcium medication was withheld experimentally for a few days and the blood sugar promptly fell off but was restored again when medication was resumed. The jaundice and digestive disturbances secondary to the lack of bile in the intestine grew progressively worse until the baby finally died.

Cases of Pre-eclamptic Toxemia and Eclampsia. Ignoring for the moment the controversies which exist regarding what factors in the abnormalities seen in eclampsia are causes and what are effects of the toxic condition, and looking superficially at the clinical picture, one is impressed with the many points of similarity in this condition and in the intoxication seen in dogs with acute liver injury. The 2 conditions have in common a more or less acute liver injury, gastro-intestinal irritation, nervous disturbances, and, as reported by Titus, Dodds, and Willets,[10] a tendency toward hypoglycemia.

Careful studies of the blood chemistry have been carried out on 8 cases diagnosed as pre-eclamptic toxemia, 4 cases of real eclampsia with convulsions, 1 case of pregnancy complicated by chronic nephritis, 1 case of pneumonia in a woman in the sixth month of pregnancy, and less complete studies on several dispensary cases of persistent vomiting of pregnancy. Several cases of normal pregnancy were also studied. Aside from the well recognized[4] tendency for the uric acid concentration in the blood to run higher than normal, the usual non-protein nitrogen constituents were found to be normal in the pre-eclamptic and eclamptic cases. The fasting blood sugar levels were definitely decreased in the pre-eclamptic toxemias—ranging between 60 and 75 mgm., as compared to concentrations of from 90 to 105 in cases of normal pregnancy. The tendency to hypoglycemia was even more marked in true eclampsia especially just before a convulsive seizure when in 2 cases blood sugar concentrations of 37 and 52 mgm. respectively were found. The guanidine concentrations were also definitely abnormal in both of the groups mentioned—values between 0.50 and 0.85 mgm. being usually obtained as compared to the normal values of from 0.25 to 0.40 mgm. per 100 cc. of blood. No abnormalities in the blood sugar or guanidine concentrations were found in the cases of pregnancy complicated by nephritis or pneumonia.

Three pre-eclamptic cases entered the hospital and were given calcium medication while the blood chemistry was carefully followed. Very promptly (within 1-2 hours) after the intravenous administration of calcium in the form of 10 cc. of a 10% calcium

[10] Titus, P., Dodds, P., and Willets, E. W., *Am. J. Obs. and Gyn.*, 1927, xiv, 89.

gluconate solution (Sandoz Co.'s sterile ampoules) and within 24 hours after the oral administration of either calcium gluconate or lactate was started the lowered blood sugar levels were restored to within the limits of 90 to 100 mgm. per 100 cc. of blood. Accompanying this change there was a general amelioration of symptoms, less nausea, and a considerable fall in blood pressure. The guanidine concentration was unaffected by medication. All 3 patients gave birth to living normal infants. Soon after delivery the guanidine concentrations returned to normal and calcium medication was no longer necessary to maintain a normal blood sugar level. Several other dispensary patients in the pre-natal clinic showed similar symptomatic improvement after calcium medication but no blood chemical studies were made.

More striking results were obtained in a case of eclampsia with convulsions given intravenous calcium medication. This patient suddenly became blind, showed tetanic symptoms, and was nearly unconscious. The blood sugar concentration was 52 mgm. and the guanidine 0.64 mgm. per 100 cc. of blood. A dose of 10 cc. of 10% calcium gluconate was given by vein. Within 15 minutes the vision was much improved and the mental condition more alert. Forty minutes after the dose the blood sugar was increased to 76 mgm. and the sight normal and the patient rational but weak. This improvement persisted for several hours. About 6 hours later it was decided to induce labor and glucose was given intravenously to improve the patient's strength. During labor, which was very long and difficult, the patient suffered a relapse into blindness and the same convulsive condition but again promptly responded symptomatically to intravenous calcium therapy. The baby was still-born. The mother survived and showed no further tendency to hypoglycemia or convulsive symptoms after delivery but died 3 weeks later from a local infection complicated with extreme anemia.

Although the number of cases studied is small, we believe that the data obtained indicate that in certain clinical types of liver disease and in eclampsia the same abnormalities in blood chemistry are present as were seen in dogs during the intoxication produced by carbon tetrachloride or chloroform. Calcium medication has furnished prompt relief in a few clinical cases showing increased guanidine and low blood sugar concentration. It is hoped that further work along these lines in laboratories where larger amounts of clinical material are available may further evaluate these findings.

We wish to thank the staffs of the Medical, Pediatrics, and Obstetrical services of the Vanderbilt University Hospital for their

kindness and cooperation in making available to us the material and cases reported in this note.

4422

Skin Reactions to Pollen and to Histamine.

ROBERT W. LAMSON.*

From the Allergy Clinic, The Los Angeles County General Hospital, Unit No. 1, California.

Certain "allergic" patients are classed as non-sensitive because no skin reactions are obtained when they are tested with the usual allergens. It is important to know if a direct relationship exists between reaction capacity and the type of skin under study, or if the site of the test is an important factor. The work of Lewis[1] and his associates has furnished us a method for studying reaction capacity in the skin hypersensitive or non-sensitive individual.

Histamine, in the following dilutions, 1-1,000; 1-10,000; and 1-100,000 (of the base) was employed as a test substance for both types of patients. Similar dilutions of pollen extracts were tested on those who were pollen sensitive. All tests were intracutaneous, and were made in the long axis on the flexor surface of the forearm. The amount injected, usually 0.01 cc., produced a welt 1.0 to 1.5 mm. in diameter. Beginning at the antecubital space the first welt was made with the 1-1,000 dilution of the test substance. The 1-100,000 was the most distal. At the wrist the opposite margin of the arm was employed and the tests were in the reverse order—the 1-1,000 was most distal. The tests were repeated 3 times using alternate arms. The wheal, the area of "reflex arteriolar dilatation",[1]—secondary erythema—was measured and the presence of pseudopods noted. The patients included some with an elastic and others with a senile or crepe-paper type of skin. Some were taking epinephrin frequently, others had never employed the drug.

The senile, atrophic, "crepe-paper" type of skin may give as large a reaction to histamine, or to pollen if the patient is sensitive, as is given by an elastic skin. The variation from patient to patient is characteristic of the individual and seemed to be unrelated to the type of skin. There were many instances where a patient gave a

* With the technical assistance of Maurine Cannon, R. N.

[1] Lewis T., *et al.*, *Heart*, 1924, xi; 1926, xiii; 1927, xiv.

similar reaction to pollen and to histamine in the 1-1,000 dilution thus suggesting that histamine may actually measure reaction capacity. The "non-sensitive" individual reacts to histamine as readily as does the sensitive one.

Twelve different patients were used in these studies, some of them being used for two different phases of the work, and in each case the tests were checked 2 or 3 times, often with an interval of a week between tests.

4423

Effect of Epinephrin Upon Skin Reactions to Pollen and to Histamine.

ROBERT W. LAMSON.*

From the Allergy Clinic, The Los Angeles County General Hospital, Unit No. 1, California.

Most "Allergists" assume that injection of epinephrin within an hour or two of the time of testing will cause false negative skin reactions. This drug is frequently given in an attempt to alleviate the symptoms of urticaria and to hasten the involution of the lesions.

Skin sensitive patients were tested as before described[1] to pollen or to histamine or to both, and non-sensitive individuals tested to the latter only. As a control the normal disappearance time of pollen or histamine wheals was determined. This factor was found to vary considerably from patient to patient but to be fairly consistent for the individual, and seemed unrelated to the test substance or type of skin.

A series of intracutaneous injections was made, measurements were taken 15 minutes later and epinephrin solution (1-1,000), 0.3 cc. (M.V.) per 150 pounds of body weight was given subcutaneously. This amount is sufficient to give a definite systemic response in most patients within 10 to 15 minutes, and to alleviate the symptoms of asthma or hay fever. Fifteen minutes after the epinephrin injection a second set of welts was made parallel to and about 1½ inches from the first set in the antecubital space and at the wrist. It was thus possible to observe the effect of this drug upon the involution of wheals already formed and the genesis of them when the test substance was injected 15 minutes after the epinephrin. The

* With the technical assistance of Maurine Cannon, R. N.

[1] Lamson, R. W., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 611.

rate of involution of wheals of pollen or histamine was little influenced by epinephrine when compared with the control rate for that individual but itching was often much less after epinephrin. This drug in the above amount did not prevent the formation of definite positive skin reactions, in fact a few patients gave as large or larger reactions after epinephrin as before. In the majority the reaction for one dilution was similar to that produced by the next higher dilution before the injection of epinephrin, thus the drug decreased the reaction about as much as would result from a 1-10 dilution of the test substance. The secondary erythema was markedly decreased by the drug in most cases.

A few tests have been made with twice the dose of epinephrin indicated above. By the use of the larger dose more marked interference with the genesis of a positive wheal was evident.

4424

The Effect of Alkalis Upon the Solubility of Quinine Salts.

A. J. HOCKETT. (Introduced by C. H. Thienes.)

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The present study was suggested by the paper by Eggleston[1] pointing out the need for further experimental data concerning factors influencing the absorption of drugs from the gastro-intestinal tract. The literature reveals a surprising dearth of material, particularly as relating to the rôle of hydrogen ion concentration as a factor in absorption.

The immediate problem was the determination of the solubility of soluble quinine salts (hydrochloride, dihydrochloride and bisulphate) in solutions of various alkaline salts at various concentrations and pH, when equal parts of 2% quinine salt and alkaline salt were mixed. The alkaline salts employed were K_2HPO_4, Na acetate, Na citrate, $NaHCO_3$ and Na_2CO_3. The effect of OH ion was learned through the use of NaOH. An end concentration of 1% quinine salt was thought to approximate the expected concentration in the stomach and duodenum after a full therapeutic dose of quinine. The pH determinations were made colorimetrically, using the

[1] Eggleston, C., *J. Am. Med. Assn.*, 1923, lxxxi, 431.

Michaelis[2] one-color series without buffers, and these values checked by the quinhydrone electrode.

TABLE I

Percentage of Alkali Used and Beginning Precipitation Points with pH Values.

Alkali	Quinine-HCl		Quinine-Di-HCl		Quinine-Bi-SO_4	
	pH	%	pH	%	pH	%
Potassium Basic Phosphate	7.07	.05	3.45	.7	3.64	.2
Sodium Hydroxide	7.12	N/300	3.40	N/10	2.89	N/150
Sodium Citrate	7.15	.02	3.64	.65	3.41	.25
Sodium Acetate	6.82	7.0	3.51	.075	3.47	9.0
Sodium Carbonate	7.71	.1	3.46	.5	3.43	.75
Sodium Bicarbonate	7.31	.3	3.67	.85	3.52	.05
Average pH Beginning PPt.	7.19		3.62		3.44	

As shown in the table, the average precipitation point of the hydrochloride is at a pH of 7.19, the dihydrochloride 3.62, and the bisulphate 3.46. It will be noted that these values are in almost the same relation as the pH values of their aqueous solutions. A 2% solution of quinine hydrochloride was found to have a pH of 6.1, dihydrochloride 2.6 and bisulphate 2.4. It will be also noted that the pH figures all lie within the pH ranges of the human stomach and duodenum, as reported by McClendon,[3] Hume, Denis, Silverman and Irwin,[4] and others.

Qualitative determinations on the precipitates showed that they were quinine salts corresponding with the precipitating alkaline salt, *viz.*, quinine acetate, phosphate, carbonate, etc. With NaOH the preciptate was quinine base. The process was apparently stoechiometric.

The results of this work so far would seem to justify the statement that physiological concentrations of alkalis definitely alter the solubility of amounts of quinine salts corresponding to the full therapeutic dose as found in the upper gastro-intestinal tract.

[2] Michaelis, Leonor, *Praktikum der physikalischen Chemie insbesondere der Kolloidchemie für Mediziner und biologen.* Berlin, 1926.

[3] McClendon, J. F., *Proc. Nat. Acad. Sci.*, 1921, xv, 347.

[4] Hume, H. V., Denis, W., Silverman, D. N., and Irwin, E. L., *J. Biol. Chem.*, 1924, lx, 633.